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PURPOSES

The main purpose of the Physiological Reviews is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology.

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THE AMERICAN
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VOL. 60 APRIL 1, 1922 No. 2

STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

XI. The Action of Cocaine and Aconitine on the Pulmonary Vagus in the Frog and in the Turtle

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From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 28, 1921

As stated in the introduction to the first report of this series (1) our knowledge of the afferent nervous mechanism of the viscera is still very incomplete. It has not been established whether stimulation of one visceral organ will continue to elicit the usual reflex response in another organ, if the afferent connection of the latter with its center has been interrupted. This research was an attempt to produce such a condition artificially. The lung was chosen as a typical viscus because complete data on the physiology of the pulmonary vagus in the frog and the turtle were collected recently by Carlson and Luckhardt (1), (4).

It might be well to recapitulate briefly the results obtained by these investigators insofar as they have a bearing on the present problem. In the frog the pulmonary vagus carries both motor and inhibitory fibers, the latter predominating and exerting a constant tonic action on the musculature of the lung. As long as the vagi are intact the lungs are kept dilated by the inhibitory impulses coming from the medulla. Destruction of the medulla or cutting the vagi causes a more or less permanent hypertonus of the lungs. Stimulation of the peripheral end of the cut vagus inhibits the lung hypertonus during the period of stimulation. In the turtle the pulmonary vagus carries mainly motor fibers. There is little evidence of any tonic activity on the part of the pulmonary vagus, except that each series of respiratory movements is

followed by a contraction of the lungs. Cutting the vagus produces no change in the lung, but the spontaneous post-respiratory contractions are abolished. Stimulation of the peripheral end of the cut vagus causes the lung to contract.

The present report comprises the results of experiments designed to elucidate the rôle of the afferent fibers of the vagus in the reflex and tonic activity of the amphibian and reptilian lung. Mechanical and electrical stimulation of the urinary bladder in the frog will ordinarily cause a reflex contraction of the lungs. The afferent pathway of the reflex arc in this case consists of the afferent fibers leading from the bladder to the spinal cord and thence by an intermediate neuron to the medulla; the efferent pathway is the pulmonary vagus. Is it possible to elicit a reflex of this nature, if no afferent impulses from the lungs could get to the medulla? Also, is the tonic inhibitory activity of the pulmonary vagus in the frog dependent upon afferent impulses reaching the center from the lungs themselves? In the turtle, will the lung whose afferent connection with the medulla has been severed contract reflexly on the inflation of the opposite lung? Will the spontaneous contractions of the lung persist under these conditions?

The greatest difficulty in experiments of this kind is in destroying or paralyzing the afferent nerve ending, or else cutting or blocking conduction in the afferent nerve fibers coming from the lung without at the same time destroying the efferent pulmonary fibers of the vagus which would of itself make it impossible for the lung to contract reflexly. Since the efferent and afferent fibers of the vagus are intermingled, it is impossible to mechanically cut one set leaving the other intact. Therefore it was thought best to employ pharmacological means, i.e., find a drug which would block the conduction in the afferent nerve fibers of the mixed nerve trunk or poison the endings of the afferent fibers in the organ itself.

The drug that suggested itself first was cocaine. It is widely employed as a local anesthetic, and while it is essentially a general protoplasmic poison, it has been shown to exert a selective action attacking the afferent nerve endings first when injected subcutaneoulsy, and the afferent nerve fibers when injected into a mixed nerve trunk as in block anesthesia. However, this selective action on afferent nerves applies only to nerves leading to the skeletal muscles. In nerves leading to visceral structures this relationship seems to be reversed. In studying the selective action of cocaine on the fibers of the vagus, Dixon (2) found that in rabbits, dogs and cats, local application of cocaine to

the vagus trunk blocked the passage of centrifugal impulses before the centripetal. This apparent difference between the "selective" effects on skeletal and visceral nerves is not due to any peculiarity of cocaine itself (which acts as a general protoplasmic poison), but rather to the difference in structure of the nerve fibers themselves. Thus in the mammal, at least, it is evident that in the skeletal nerves the centrifugal, and in visceral nerves the centripetal fibers are more resistant to the action of cocaine.

Another interesting observation was made by Kast and Meltzer (3). These investigators found that the sense of pain normally present in the visceral organs of dogs was completely abolished by subcutaneous injection of cocaine such as made by surgeons who perform abdominal operations under local cocaine anesthesia. They ascribed this phenomenon to the absorption of cocaine into the circulation. This would indicate that the nerve endings of the visceral nerves are more sensitive to cocaine than those of the skeletal nerves.

Aconitine was also made use of. It and its allies are the only drugs which when injected intravenously will poison the endings of afferent nerves. For this reason it would be especially useful for attempting to reach the afferent nerve endings by way of the circulation.

METHODS: *Frogs.* The contractions of the lung of the frog were recorded by inserting a cannula into the tip of the lung, and connecting it with a water manometer or a sensitive tambour. The animals were always decerebrated, and prepared according to the method developed by Carlson and Luckhardt (1). For local application of drugs each lung was insulated by means of sheet rubber so that no drug could get to the neighboring viscera. Drugs were injected intravenously through a cannula inserted into the median abdominal vein. To prevent a drug injected intravenously from reaching one of the lungs used as a control the pulmonary artery on that side was ligated. In such cases methylene blue was injected at the end of the experiment, and the absence of blue in the corresponding lung was used as a test of the efficacy of the method. It may be added that as a general procedure only one vagus was used, the other being ligated and the lung on that side allowed to go into hypertonus. This was an indication that the preparation was in good physiological condition. Under certain conditions the tonic inhibitory activity of the pulmonary vagi seems to be entirely abolished, so that cutting these nerves or destroying the medulla has no effect on the lungs whatever. A large number of control experiments was necessary for this reason.

Turtles. As a rule one lung (the left) was isolated and used for recording lung contractions, the other being left in communication with the outside, through a tracheal cannula, for the purposes of respiration. As in the case of the frog the procedure of preparing the decerebrated animal for lung contractions was that of Carlson and Luckhardt (4). The lung contractions were recorded by a sensitive tambour or by a water manometer. For the local application of cocaine both vagi were isolated in the neck for a length of about 2 inches. A piece of cotton about the size of a small pea was saturated with the solution of the drug, the middle portion of the isolated nerve was insulated with a strip of sheet rubber, the cotton placed on the nerve, and the rubber tied with a thread, tight enough to express the solution on the nerve, but not so tight as to compress the nerve itself. The stimulating electrodes were then applied to the proximal or distal portion of the nerve, in either case about 1 cm. away from the place where the drug was applied. For intravenous injections the external jugular vein was cannulated. In the turtle also the presence of the normal spontaneous lung contractions depended on outside conditions. During the second half of June and the very last week of August all turtles used showed spontaneous contractions of the lungs as soon as the preparations were ready for recording. During July and the greater part of August not a single turtle could be found to exhibit this phenomenon.

EXPERIMENTS WITH COCAINE ON FROGS: *Intravenous injections of cocaine.* Before any injection was made the reflexes were tested. The urinary bladder and a loop of the intestine were stimulated mechanically or electrically, and a reflex response elicited from the lungs and the heart. The lungs contracted and the heart was slowed or stopped entirely. Various amounts of cocaine were then injected into the median abdominal vein, and the reflexes tested after each injection. One-tenth milligram cocaine hydrochloride in 1 cc. Ringer's when given by vein, never had any effect on the visceral reflexes tested, but it caused a slight and fleeting contraction of the lung. The same result was obtained with 0.2 mgm. cocaine (fig. 1). The injection of 0.5 mgm. cocaine in 0.5 cc. Ringer's caused a very marked contraction of the lung, with a short-lasting abolition of the lung reflexes. The effect of these doses is of very brief duration. The temporary failure to elicit a reflex may be due to the injury to the center, the efferent or or afferent terminus of the reflex arc, or to both. It will be shown presently that small doses of cocaine have no effect on the center in the medulla. Whether the afferent nerve endings in the viscera were

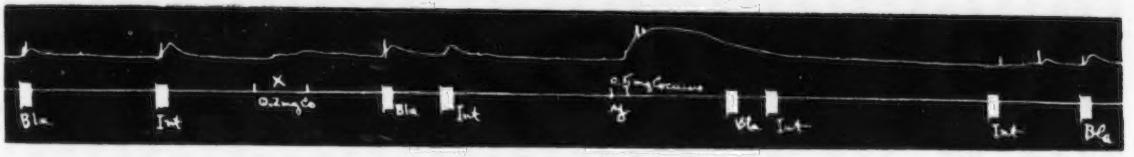

Fig. 1. Water manometer tracing of the reflex contractions of the left lung of the frog following stimulation of the bladder, *Bla*, and intestine, *Int*. Secondary coil at 10 cm., duration of stimulation 10 seconds. Effect of the intravenous injection of 0.2 mgm. *x* and later 0.5 mgm. *y* cocaine hydrochloride on the lung musculature and the lung reflexes. Showing temporary hypertonus of the lung and abolition of reflexes into the lung following intravenous injection of cocaine with return of the normal inhibitory tonus of the lung and lung reflexes as the action of the drug wore off.

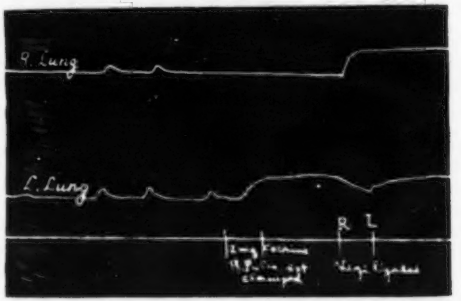

Fig. 2. Upper tracing is a tambour record of the intrapulmonic pressure of the right lung of a frog; lower tracing is a water manometer record of the pressure in the left lung. Right pulmonary artery clamped, and 2 mgm. cocaine injected into the median abdominal vein. *R* = right vagus ligated; *L* = left vagus ligated. Showing that the hypertonus of the lung following the intravenous injection of moderate doses of cocaine is due to a peripheral and not a central action of the drug.

poisoned could not be determined. Stimulation of the mesentery which contained nerve fibers (never affected by a poison in circulation) likewise failed to produce a reflex contraction of the lung. This would indicate that the efferent inhibitory fibers of the vagus were temporarily paralyzed or depressed. The contraction of the lung which becomes more marked as the dose of cocaine is increased, and eventually simulates the effect produced by cutting the vagus, confirms this supposition. It may be added that the inhibitory endings of the vagus in the lung showed approximately the same resistance to the action of cocaine as the inhibitory endings in the heart. Sometimes after the injection of 0.5 mgm. cocaine the heart reflexes disappeared while those of the lung persisted, and sometimes the opposite was true. The injection of 1 mgm. cocaine or more abolished both the lung and the heart reflexes invariably, and for a long time. In either case the efferent vagus terminations were paralyzed. In the lung cocaine produced the characteristic hypertonus that ensues on ligation of the vagus. That the so-called "escape" of the lung from the inhibitory control of the vagus was of purely peripheral origin was shown by the following experiment (fig. 2). The right pulmonary artery was clamped off, and the blood vessels in the right lung gradually disappeared. Two milligrams of cocaine were then injected intravenously. The left lung went into a condition of hypertonus, but the right lung was not affected, thus showing that the center in the medulla was not paralyzed. Ligation of the right vagus caused the usual "escape" of the right lung, confirming the fact that the center continued to send tonic inhibitory impulses after the injection of cocaine. In the meantime the effect of cocaine on the left lung was beginning to wear off, and the lung was gradually dilating. At this point ligation of the left vagus caused the lung to contract again, but it will be noticed this contraction was of the same magnitude as that caused by the cocaine. This shows that the previous contraction was a true "escape" from vagus inhibition caused by the paralysis of the inhibitory endings of the vagus in the lung.

Stimulation of the vagus after the intravenous injection of cocaine produced no relaxation of the contracted lung, if the induced current was not very strong. With very strong currents inhibition of the lung could be obtained even after 2 mgm. cocaine were injected. With larger doses of cocaine (5 to 8 mgm.) even the strongest current produced no dilatation of the lung, nor slowing of the heart. This would indicate that the efferent inhibitory endings were not actually paralyzed by the

smaller doses of cocaine, but only depressed so that the ordinary tonic inhibitory impulses from the medulla could not reach the lungs (thus producing hypertonus). Strong stimuli, however, could "break through" the depression, and so produced dilatation of the lung.

Intravenous injection of cocaine after the ligation of the vagi was frequently without any further effect. Occasionally large doses of cocaine produced a further contraction, apparently by direct action on the peripheral mechanism.

In several experiments the skeletal reflexes were tested along with the visceral. The central end of the cut sciatic nerve of one leg was stimulated with a very weak induced current, and it was noticed that even large amounts of cocaine did not abolish the contralateral reflexes. If anything, the skeletal reflex excitability was increased, a well-known effect of cocaine. Thus, it is evident that the efferent endings of the skeletal nerves are much more resistant to the action of cocaine than the efferent endings of visceral nerves.

Intra-arterial injections of cocaine. It was interesting to determine the dose of cocaine which when injected intravenously would cause an "escape" of the lungs from vagus inhibition by paralyzing the center in the medulla. It will be recalled that 1 to 2 mgm. cocaine produce this effect by purely peripheral action. Prior to injecting the drug both pulmonary arteries were clamped or ligated, to prevent the cocaine from getting to the endings of the vagus in the lungs. As larger amounts of cocaine were injected the effect on the heart interfered with the experiments. The heart would stop, and the drug would get to the lungs by way of the pulmonary veins. Under these circumstances the lungs contracted, but this was due to the peripheral effect of the drug, and the effect on the center, if any, was masked. That this was the case was shown by the fact that methylene blue injected intravenously, with the heart at standstill, found its way to the lungs, in spite of the ligation of the pulmonary arteries.

It may be pertinent to record some observations on the action of cocaine on the heart in situ. Small amounts (1 to 2 mgm.) injected intravenously merely slowed the heart temporarily. Larger doses or repeated injections of small doses often produced complete arrest of cardiac action, the ventricle being enormously dilated and engorged with blood. In some cases complete heart block was observed; this gradually changed into 6:1 rhythm, then to 5:1 rhythm, and so on, until the heart recovered completely. So far as I am aware, this effect was never reported before. However, large amounts of cocaine could

be injected very slowly without causing the heart to stop even temporarily. Thus into one frog 7 mgm. cocaine were injected, and forty minutes later another injection of 7 mgm. was made, without causing the heart to stop. In each case the heart was slowed considerably, and "peristaltic" movements of the ventricle were observed.

This action of cocaine on the heart made it impossible to paralyze the vagus center by intravenous injections. At the suggestion of Doctor Luckhardt, the following procedure was employed with success. Both pulmonary arteries were ligated. A cannula was inserted into the left aortic branch, the mouth of the cannula being directed toward the heart. The cocaine injected through this cannula was driven first toward the heart, but at the bifurcation of the aorta it mixed with blood and was carried to the medulla. This could actually be seen through the walls of the vessels, and if the injection was made slowly, no cocaine would get into the heart. Apparently no excessive pressure was developed in the aorta, and the effect on the medulla could not have been mechanical.

By means of this method various amounts of cocaine had to be injected to paralyze the vagus center, the average dose being 5 mgm. As the drug was given unilaterally this dose corresponds to 10 mgm. cocaine given intravenously. Subsequent injection of methylene blue gradually caused the entire body of the frog, the lungs excepted, to be colored with the dye, thus confirming the fact that the action was central.

It will be noted that a dose of cocaine sufficient to paralyze the endings of the vagus in the lungs will not paralyze the center in the medulla showing that the cells in this case are more resistant to the action of cocaine circulating in the blood than the axone terminations. This fact is not in agreement with the general conception regarding the action of cocaine. Thus Cushny in his *Text-book of Pharmacology* (5), speaking of cocaine, says that "if brought in contact with other forms of living matter in the concentration used in anesthetizing nerve ends, it is poisonous to all structures which have been examined. Even concentrations too low to act on the peripheral nerves act on the nerve cells and paralyze them. . . . ." It must also be remembered that selective action of a general protoplasmic poison merely indicates a difference in structure in the various forms of protoplasm acted upon. It must be concluded, therefore, that the efferent endings of the visceral nerves differ greatly from the endings of skeletal nerves in their resistance to the action of cocaine. This conclusion, if correct, would

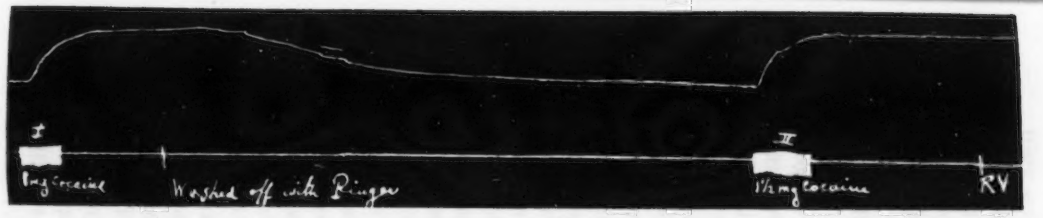

Fig. 3. Water manometer record of the intrapulmonic pressure of the right lung of a frog. *I* = 1 mgm. cocaine in ½ cc. Ringer's applied to the surface of the lung producing a marked contraction. The lung relaxed when the cocaine was washed off. *II:* A second application of 1½ mgm. cocaine produced a similar effect. Subsequent ligation of the vagus at *R.V.*—no further contraction. Showing the prompt but temporary hypertonus of the lung is due to a depression or paralysis of the inhibitory nerve endings of the vagus in the lung when cocaine is applied locally to the lung in moderate doses.

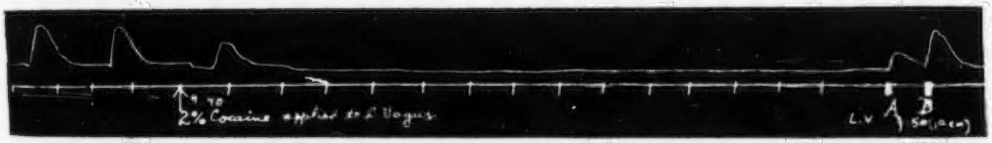

Fig. 4. Water manometer tracing of the spontaneous contractions of the turtle's lung. Signal marks indicate respiratory movements. Application of a 2 per cent cocaine solution to the vagus trunk abolished spontaneous contractions of the lung. Respiration continued. Stimulation of the vagus above, *A*, and below, *B*, the point where the drug was applied produced contraction of the lung.

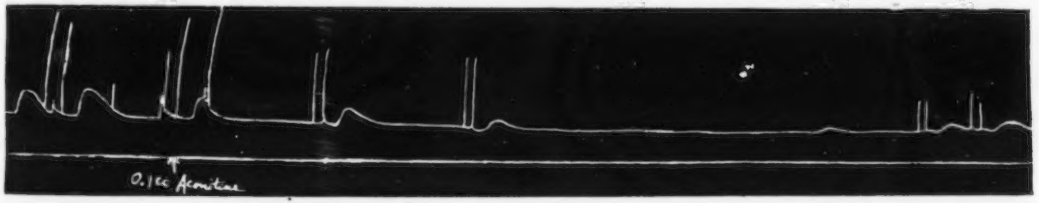

Fig. 5. Water manometer tracing of the intrapulmonic pressure of the turtle's lung. Five minutes after the intravenous injection of 0.1 cc. of a saturated solution of aconitine, respiration stopped and the spontaneous lung contractions disappeared at the same time. Eight minutes later both respiration and spontaneous lung contractions returned.

explain the results obtained by Kast and Meltzer (3) who found that in local cocaine infiltration anesthesia of the abdominal area pain sensations from the viscera are entirely abolished. They held that some cocaine was absorbed into the circulation, and that this small amount of cocaine paralyzed the endings of the pain fibers in the viscera, while it was unable to produce general body anesthesia. In the light of the above-mentioned facts such an explanation seems to be very plausible, as it is based on the assumption that the visceral nerve endings offer a very low resistance to the action of cocaine.

Local application of cocaine. When 0.1 mgm. cocaine in 1 cc. Ringer's is applied to the surface of the frog's lung, a slight and transient contraction is produced. With larger amounts of cocaine the contractions are more marked and more lasting. One milligram cocaine in 1 cc. Ringer's will produce a maximal contraction of the lung (fig. 3). If the cocaine is washed off the lung will relax and gradually return to its original size. A second application of an equal amount of cocaine will produce precisely the same effect, and this can be repeated many times. If the vagus is ligated shortly after the application of cocaine (fig. 3) no further contraction is produced showing that the cocaine paralyzed the nerve endings and that the contraction was maximal. Stimulation of the peripheral part of the ligated vagus will not cause the lung to relax, unless the current is very strong. If the cocaine applied to the lung is not washed off, the lung will relax nevertheless, but only after a considerable interval of time. This is apparently due to the absorption of the cocaine into the circulation, as in this case a second application of cocaine or ligation of the corresponding vagus will produce a maximal contraction of the lung. To confirm the conclusion that local application of cocaine will temporarily depress or even paralyze the efferent inhibitory endings of the pulmonary vagus use was made of the nicotine method. Carlson and Luckhardt (1) have shown that after the vagus in the frog was ligated, and the corresponding lung "escaped" from its tonic inhibitory control, the injection of nicotine effected a marked dilatation of the lung by stimulating the efferent inhibitory endings of the pulmonary vagus. The following experiment was performed. The right vagus was ligated producing hypertonus of the right lung; 2 mgm. cocaine (1 cc.) were applied to the left lung producing a similar hypertonus, and subsequent ligation of the left vagus had no further effect. One milligram nicotine in 1 cc. Ringer's was now injected into the median abdominal vein, and this produced an immediate inhibition of the right lung, the left lung remaining unaffected.

When a 2 per cent solution of cocaine was applied to the vagus directly, the corresponding lung contracted markedly. Subsequent ligation of the vagus produced no further contration of the lung; nor did stimulation of the vagus above the point of application of the drug cause inhibition of the lung, unless the current was very strong. This shows that local application of cocaine will abolish conductivity in the fibers of the vagus.

EXPERIMENTS WITH COCAINE ON TURTLES: *Intravenous injections of cocaine.* As little as 0.5 mgm. cocaine hydrochloride in 1 cc. Ringer's, when injected into the external jugular vein, may abolish the spontaneous post-expiratory contractions of the turtle's lung for one hour. This, however, is a rare occurrence. Ordinarily 3, 4 or 5 mgm. cocaine must be injected to abolish these contractions for a like period of time. The length of time required for complete recovery was not exactly proportional to the dose given, but depended also upon the physiological condition of the preparation. The lung reflexes were abolished together with the spontaneous contractions, and returned with them. Stimulation of the vagus produced no contraction of the lung, unless the current was very strong. It will be noticed that the effect of cocaine injected intravenously on the endings of the motor fibers of the vagus in the lung of the turtle is essentially the same as that on the endings of the inhibitory fibers of the vagus in the lung of the frog. The skeletal reflexes were not suppressed even by the largest doses of cocaine (up to 20 mgm.), showing that, as in the frog, the efferent endings of the skeletal nerves are more resistant to the action of cocaine than the efferent endings of the vagus.

The heart could not be observed directly, but the pulse in the arteries on the surface of the lung served as a criterion of the condition of the ventricle. In the doses used cocaine slowed the pulse temporarily. In one turtle the pulse disappeared for fifteen minutes after the injection of 8 mgm. cocaine.

Application of cocaine to the trunk of the vagus. Weak solutions of cocaine (0.5 per cent) applied to the vagus in the neck have no effect on the conductivity of its fibers. The spontaneous contractions of the lungs are not abolished. Stronger solutions of cocaine, 2 to 5 per cent, will temporarily abolish the spontaneous contractions, an effect usually following the division of the vagus. This would indicate that cocaine blocked the conduction in the efferent fibers of the vagus. To test this, the vagus was stimulated electrically centrally and peripherally to the point where the cocaine was applied. While stimulation below

that point always produced a very marked contraction of the lung, stimulation above it produced no contraction, or a feeble contraction, when the concentration of the cocaine solution was low, and the current very strong. Upon the removal of the drug and repeated washing of the nerve with Ringer's solution the conductivity of the fibers gradually returned. Stimulation of the vagus above the point where the cocaine was applied produced stronger and stronger contractions of the lung, although the strength of the current and the duration of stimulation remained constant. The spontaneous contractions of the lung returned also. The degree of depression was greater the higher the concentration of the cocaine solution applied. The adherents of the "all-or-none" theory as regards the conduction of nerve impulses would say that the record shown in figure 4, where stimulation above the point of application of cocaine produced a feebler contraction of the lung than stimulation below that point, merely indicates that only the outside fibers of the vagus were affected by the 2 per cent cocaine solution, and contraction *A* was a result of stimulation of a fewer number of fibers (the inside fibers not affected by cocaine). This explanation would make it unnecessary to assume that there was a depressed conductivity in all the fibers. But it would be difficult to explain why the normal impulse following each series of respiratory movements failed to reach the lung and produce a contraction. Obviously, if the inside fibers were unaffected, some contraction of the lung, though possibly a very feeble one, should follow the respiratory movements. Since the spontaneous contractions disappeared entirely, it seems more probable that cocaine *depressed the conductivity in all the fibers* of the vagus. Apparently the normal periodic stimuli sent along the vagus from the lung-motor center were blocked by the depressed conductivity of the fibers of the vagus. A weak electrical stimulus was also blocked, while a strong electrical stimulus would pass in spite of the depressed state of the fibers. And that is what was actually observed.

Experiments were also performed to detect a difference, if any, in the effects produced by the local application of cocaine on the efferent and afferent fibers of the vagus. While the results obtained were not uniform, it may be stated that the afferent fibers are more resistant to the action of the drug than the efferent fibers. The procedure employed was as follows. The left lung was connected with a water manometer. Both vagi were isolated. Stimulation of the right vagus produced a reflex contraction of the left lung. A 5 per cent solution of cocaine was applied to the right vagus, and the nerve stimulated cen-

trally and peripherally to the point where the drug was applied. In about 30 minutes conduction in the afferent fibers was blocked, and stimulation of the right vagus peripherally to the point of application of cocaine failed to elicit a reflex contraction of the left lung. Washing the cocaine off the vagus would sometimes restore the conductivity of its afferent fibers, but only after several hours. As a rule, cocaine was applied next to the left vagus of the same turtle. The conductivity of the efferent fibers was abolished in less than ten minutes, and stimulation of the vagus centrally to the point of application of cocaine produced no contraction of the lung. Washing the cocaine off the left vagus ordinarily restored the conductivity of its efferent fibers in an hour or two, and in one case in about ten minutes. It is evident that the efferent fibers of the vagus are much less resistant to the action of cocaine than the afferent fibers.

EXPERIMENTS WITH ACONITINE ON FROGS: According to the U. S. Pharmacopeia, aconitine is almost insoluble in water. For use in these experiments a few crystals of the drug were shaken up with a large quantity of Ringer's solution, and after the crystals settled on the bottom the supernatant liquid was poured off and used as a saturated solution of aconitine. It must be borne in mind that on account of its extreme insolubility a "saturated" solution of aconitine may be likened to a saturated solution of silver chloride or barium sulphate; in other words, though saturated, it is still extremely dilute. No attempt was made to determine the exact concentration of the solution, as the drug very often contains impurities, aconine and benzaconine, and its activity varies considerably. Some samples are one hundred times stronger than others. To assay roughly the potency of the aconitine used, various amounts of the saturated solution were injected into frogs. It was found that 0.3 cc. of aconitine injected into the ventral lymph sac will paralyze a frog weighing 35 grams in about forty minutes, and kill it in one and a half hours. Fibrillary twitching of the skeletal muscles and strychnine-like convulsions preceded the paralysis. The motor endings of the skeletal nerves preserve their irritability, as stimulation of the sciatic nerve produced a contraction of the gastrocnemius.

Intravenous injections of aconitine. When injected intravenously, $\frac{1}{20}$ or even $\frac{1}{50}$ cc. of the saturated solution of aconitine will cause an "escape" of the lungs from vagus inhibition, by paralyzing the efferent inhibitory endings of the pulmonary vagus. In order to show that this action was local, the same method was used as in experiments with cocaine. The right pulmonary artery was clamped, and the injection of aconitine produced no contraction of the right lung,

although the left lung contracted immediately. Subsequent ligation of the right vagus produced an escape of the right lung from vagus inhibition, showing that the center continued to exert its tonic inhibitory activity after the injection of aconitine.

Intra-arterial injections of aconitine. To determine the dose of aconitine that would produce a contraction of the lungs by paralyzing the vagus center in the medulla larger quantities of the drug had to be injected, and its action on the heart, such as reversal of rhythm, made it necessary to use intra-arterial injections. The procedure and control were the same as employed in the intra-arterial injections of cocaine. It was found that 0.1 cc. of the saturated solution of aconitine when administered in this way will paralyze the vagus center and cause the lungs to contract. This dose corresponds to an intravenous dose of 0.2 cc.

Local application of aconitine. Variable results were obtained with this method. However, the dose necessary to paralyze the endings of the vagus by local application was very large. Sometimes 1 cc. of the saturated solution (three times the lethal dose when injected into the ventral lymph sac) had to be applied to the lung to produce an "escape" from vagus inhibition. Very often peculiar spontaneous rhythmic contractions of the lungs would appear after the local application of aconitine, and each lung showed a rhythm of its own. It is possible that aconitine stimulates the local nervous mechanism, while paralyzing the inhibitory endings of the vagus.

EXPERIMENTS WITH ACONITINE ON TURTLES: The intravenous injections of very small amounts of aconitine were without effect. But when the dose was increased to 0.1 cc. of the saturated solution, aconitine abolished the respiratory movements, and the spontaneous lung contractions that follow them, in about five minutes (fig. 5). Both respirations and lung contractions returned in eight minutes; they were feeble at first, but soon regained their original magnitude. The intravenous injection of 0.2 cc. aconitine abolishes respiratory movements and spontaneous lung contractions for four or five hours, but when respiration was restored, the lung contractions were invariably restored with it. Stimulation of the vagus before and after the injection of aconitine produced lung contractions of exactly the same magnitude, showing that the efferent nerve endings were not paralyzed. Permanent cessation of respiration and disappearance of spontaneous lung contractions were observed after the injection of 0.5 cc. of aconitine. The action was central, as similar results were obtained when the pulmonary artery was ligated. Moreover, with the lung circulation intact, stimu-

lation of the vagus after the injection of 0.5 cc. aconitine produced a contraction of the lung. Stimulation of the opposite vagus also produced a contraction of the lung (reflex) indicating that the lung-motor center was intact. The only conclusion possible is that the respiratory center was paralyzed by aconitine. A very characteristic phenomenon was the contracture of the lung after the injection of aconitine (fig. 6). Subsequent stimulation of the vagus on the same or opposite side produced an added contraction of the lung, without relaxation. In some experiments this contracture did not occur spontaneously, but only after one of the vagi was stimulated.

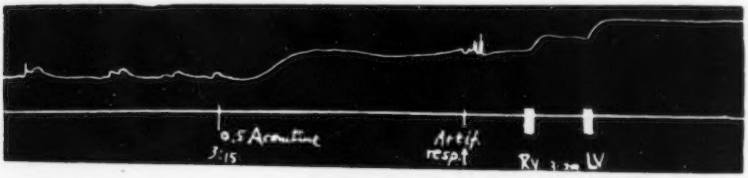

Fig. 6. Tambour record of the intrapulmonic pressure of the left lung of the turtle. Intravenous injection of 0.5 cc. of aconitine abolished respiration and spontaneous lung contractions. The lung went into a state of contracture. Stimulation of the right and left vagus produced further contractions of the lung, but no relaxation followed these contractions. Drum moving very slowly.

The pulse in the lung arteries was weakened and slowed by the intravenous administration of aconitine.

The lethal dose of aconitine for the turtle varied between 2 and 5 cc. of the saturated solution.

SUMMARY

1. The intravenous injection of 1 mgm. cocaine hydrochloride will abolish the lung and heart reflexes in the frog by paralyzing or greatly depressing the efferent endings of the vagus. The lung "escapes" from the inhibitory control of the vagus for the same reason. Whether the afferent endings are similarly affected has not been established.

2. A dose of cocaine sufficient to paralyze the efferent endings of the vagus is without effect on the efferent endings of the skeletal nerves.

3. A much larger dose of cocaine is necessary to produce an "escape" of the lungs by paralyzing the vagus center. As a general protoplasmic poison, cocaine is held to be more poisonous to the cells of the central nervous system than to peripheral nerve endings, but in the vagus, at least, the cells are more resistant to the action of the drug than the efferent nerve endings.

4. Intravenous injection of cocaine will produce slowing of the heart, if the dose is small (1 to 2 mgm). Larger doses cause complete or partial heart block, and sometimes "peristalsis" of the ventricle.

5. Local application of cocaine to the lung of the frog has the same effect as the intravenous injections of the drug.

6. Intravenous injection of cocaine will abolish the spontaneous lung contractions in the turtle by paralyzing or depressing the efferent motor nerve endings of the pulmonary vagus, essentially the same effect as that observed in the frog.

7. The spontaneous lung contractions in the turtle disappear after a 2 to 5 per cent cocaine solution had been applied directly to the vagus trunk in the neck. Apparently the efferent impulses from the lung-motor center are blocked by the cocaine. Stimulation of the vagus above the point of the application of the drug produces a feebler contraction of the lung than stimulation below that point. This is not due to blocking the conduction in some fibers, but rather to a depression of the conductivity in all the fibers.

8. The afferent fibers of the pulmonary vagus are more resistant to the action of cocaine than the efferent fibers. A similar result was obtained by Dixon on the vagus of the dog, the cat and the rabbit.

9. Minute doses of aconitine will paralyze the efferent inhibitory endings of the pulmonary vagus in frogs, and larger doses will paralyze the vagus center. This resembles the action of cocaine on the same structures.

10. Intravenous injection of aconitine will paralyze the respiratory center in turtles, and thus indirectly abolish the spontaneous contractions of the lungs. Aconitine also produces a state of contracture in the turtle's lung.

It is a pleasure to acknowledge my indebtedness to Dr. A. J. Carlson for suggesting this study, and to Dr. A. B. Luckhardt for his constant aid in overcoming the technical difficulties and for his numerous suggestions as to the control of the experiments.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

XII. The Response of the Isolated Esophagus of the Frog and the Turtle to Certain Drugs

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It was reported by Carlson and Luckhardt (1) that in the turtle stimulation of the peripheral vagus causes inhibition of the circular neuro-muscular mechanism of the upper two-thirds of the esophagus and contraction of the longitudinal system in the region of the cardia. This would seem to indicate a difference in innervation of the esophagus at different levels. These investigators also noted that the hypertonus of the frog's esophagus following vagi section and vagi stimulation seemed to be confined principally to the longitudinal system.

The present study was undertaken at the suggestion of Doctors Carlson and Luckhardt with the view of ascertaining whether further light on the motor control of the esophagus might be secured by the study of the reaction of isolated preparations of the esophagus to the so-called neurotropic and myotropic drugs.

Methods: The esophagus was prepared for studying the circular and longitudinal neuro-muscular systems in the following manner:

1. Frog. In the experiments on the frog the entire esophagus was used. In studying the circular system the esophagus was suspended as a ring in a muscle warmer and connected with German silver wire hooks to a delicate heart lever which recorded on a slowly moving drum. After the frogs were pithed and the esophagus exposed usually less than one-half a minute was required to cut it off at the cardia and pharynx and suspend it in the bath.

It was found necessary to turn the esophagus inside out before any dependable reactions to the drugs could be obtained. With the mucous membrane in its normal position, that is, inside, the circular muscle fibers and their nervous mechanism seem to be almost completely inaccessible to the drugs in solution in the bath.

In the study of the longitudinal neuro-muscular mechanism the whole esophagus again was used. One end was attached by means of a small clamp to a fixed point in the muscle warmer and the other by a similar clamp by a silk thread to a delicate heart lever. The weight of the clamps was carefully counterbalanced.

The fluid in the muscle warmer was changed by displacing it from below, thus preventing exposure of the tissue to the air.

2. *Turtle.* After pithing the turtle and removing the plastron the entire esophagus and stomach were carefully cut away from their surrounding tissues. In studying the circular system two methods of suspending the preparations were used. First, the same method as used in studying the circular fibers of the frog esophagus. Another method was to use the small clamps. These were applied to the esophagus so that the direction of work of the muscle was the same as when it was used as a ring. The whole ring was used in this case also.

The clamps were used entirely when studying the longitudinal system, strips of about 1 cm. in length being used.

In all the work on turtle tissues two or three rings or strips were usually suspended in the same bath. This was accomplished by using a piece of glass rod for a holder and having the clamps or hooks attached to it. This was dipped into a container made from an inverted bottle from which the bottom had been cut away. In this case the fluid was drained from the bottom of the container and the fluid to replace it was then poured in promptly. The tissues in this case were thus exposed to the air for about five seconds at each change of the bath. This was carefully checked by repeated changes with the Ringer's solution. It was found that no appreciable changes followed this short exposure to the air. In all the work the fluids were used at room temperature.

RESULTS ON THE FROG ESOPHAGUS: 1. *Need of oxygen for the spontaneous activity of the circular mechanism.* Early in the work it was noted that when the preparation was immersed in a non-oxygenated Ringer's solution there was an inhibition of the spontaneous contractions and the tone of the isolated ring of esophagus (see fig. 1, *C*).[1] This inhibition did not occur if oxygen had been previously bubbled through the Ringer's solution (fig. 1, *C*).

When the ring of the esophagus is isolated and suspended in oxygenated Ringer's solution only a few minutes are as a rule required before the development of a vigorous rhythm. If the ring be suspended

[1] The tracings in this article are reduced about two-thirds.

in non-oxygenated Ringer's solution there may or may not be a few spontaneous contractions followed by hypertonus (see fig. 1, *A*). Addition of oxygen to the bath with the preparation in this condition is followed by an increase in tone and a vigorous spontaneous activity in the ring (fig. 1, *A*). If in an active preparation the oxygen be shut off and non-oxygenated Ringer's solution be placed in the bath there is inhibition of the contractions and tone and finally complete cessation

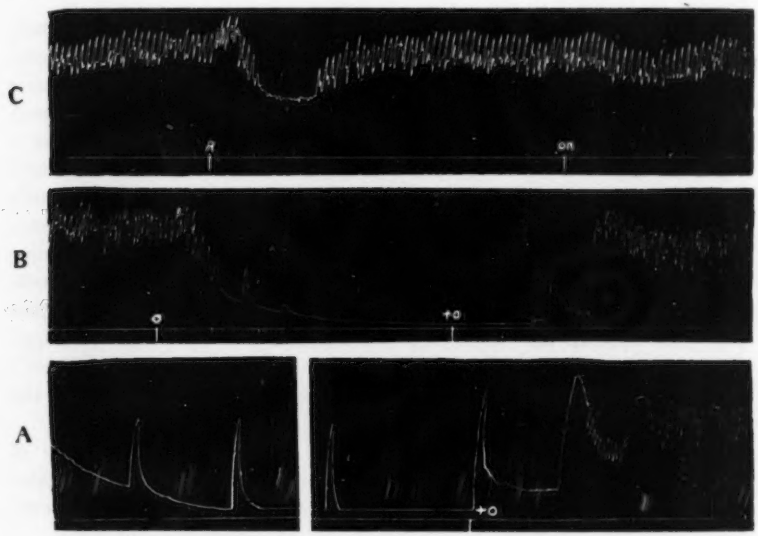

Fig. 1. Frog esophagus. Record of isolated circular neuro-muscular system. A; suspended in Ringer's solution without oxygen; showing complete hypotonus: $+O$, start bubbling oxygen through bath, showing increased tone and development of spontaneous rhythm. B; active spontaneous contractions in oxygenated Ringer's solution; $-O$, shut off oxygen and replaced bath with non-oxygenated Ringer's solution; showing decrease in tone and failure of contractions: $+O$, replaced oxygen, showing recovery of tone and contractions. C; active spontaneous contractions in oxygenated Ringer's solution. *R*, changed bath with non-oxygenated Ringer's solution; showing temporary inhibition. *OR*, changed bath with oxygenated Ringer's solution and showing no effects on the preparation.

of all spontaneous activity (fig. 1, *B*). With the ring in this condition the addition of oxygen to the solution is again promptly followed by spontaneous contractions and increased tone to the previous level (fig. 1, *B*). *This indicates that oxygen is necessary for the spontaneous activity of the isolated ring of frog's esophagus.*

The inhibition in non-oxygenated Ringer's solution was not as great in preparations which had been previously inverted. In view of this influence of non-oxygenated Ringer's solution on the spontaneous activity of the isolated ring, in all subsequent work on this type of preparation the precaution was taken of using only oxygenated Ringer's solution in making up the dilutions of the drugs to be studied. The longitudinal mechanism does not seem to be as sensitive as the circular system to changes in the oxygen supply.

When the isolated ring of esophagus is suspended in a bath through which air is bubbled instead of oxygen, a poor and irregular spontaneous activity develops for a short time. The tone is low and the contractions are far apart with an early failure. If at this time oxygen be substituted for the air there is a prompt increase in tone and the development of a vigorous rhythm. In the active preparation replacing oxygen with air or changing the bath to a non-oxygenated Ringer's solution is soon followed by complete failure of the contractions and a drop in tone. The substitution of oxygen for air or for non-oxygenated Ringer's solution is followed by a restoration of tone and contractions. As a rule no inhibition occurs if the bath be changed with Ringer's solution through which air has been bubbled for a long time (one hour or more). If in the active preparation the bath be changed with aerated Ringer's solution and air be substituted for oxygen there is a cessation of the spontaneous contractions and complete inhibition of tone.

2. Adrenalin. In 14 experiments there was an increased tone of the circular ring following the administration of adrenalin in a dilution of 1:100,000 (fig. 2, *A*). This increase was prompt in its occurrence and always marked. The recovery was slow.

In 7 experiments an abrupt and profound inhibition of both tone and contractions followed adminstration of the same quantity of adrenalin (fig. 2 *B*). Whichever reaction was obtained it could always be repeated on the same preparation. It was never possible on the same preparation to obtain stimulating and also inhibiting effects with adrenalin. Both adrenalin reactions were obtained after atropine.

Addition of adrenalin to the bath was always followed by an inhibition of the longitudinal system (fig. 3, *A* and *B*). This inhibition consisted of both a drop in tone and also cessation of the spontaneous contractions. This inhibition always came on promptly and usually remained until the drug was removed from the bath. Recovery was also prompt. The inhibitory effect of adrenalin is more marked after atropine than before, in the same preparation (fig. 3, *B*). Repeated

administrations of adrenalin in the same preparation are followed by decreased reactions.

It was found that a definite inhibition of the longitudinal system could usually be obtained with adrenalin in dilutions up to 1:30,000,000. In some sensitive preparations the reaction was obtained with a dilution of adrenalin of 1:100,000,000 (fig. 3, *A*). The influence of atropine on the reaction to this dilution of the drug was not tested out.

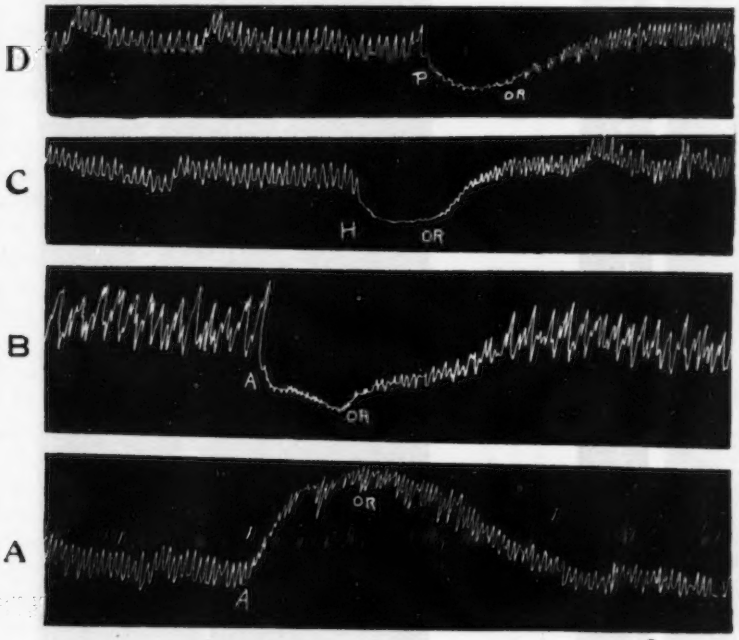

Fig. 2. Frog esophagus. Record of isolated circular neuro-muscular system in oxygenated Ringer's solution. *OR*, changed bath with oxygenated Ringer's solution. A: showing the stimulating influence of adrenalin, *A*; B: the inhibition in another preparation from the same quantity of adrenalin (1:100,000); C; the inhibitory influence of histamine, *H*; D: the inhibition after pituitary liquid, *P*.

3. Pilocarpine. Stimulating effects always follow the administration of pilocarpine to preparations of the frog's esophagus in dilutions of 1:100,000. These are the same for both circular and longitudinal systems and include a prompt and abrupt rise in tone. After a short

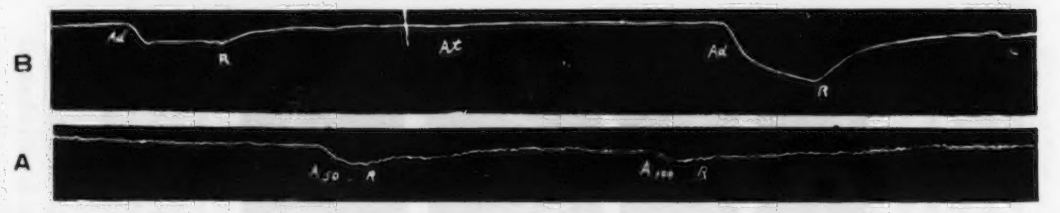

Fig. 3. Frog esophagus. Record of longitudinal neuro-muscular system in oxygenated Ringer's solution. A: showing inhibition caused by adrenalin in dilutions of 1:50,000,000, *A-50*, and 1:100,000,000, *A-100*. B: showing inhibition caused by adrenalin, *Ad*, in dilution of 1:100,000 before and after atropine, *At*.

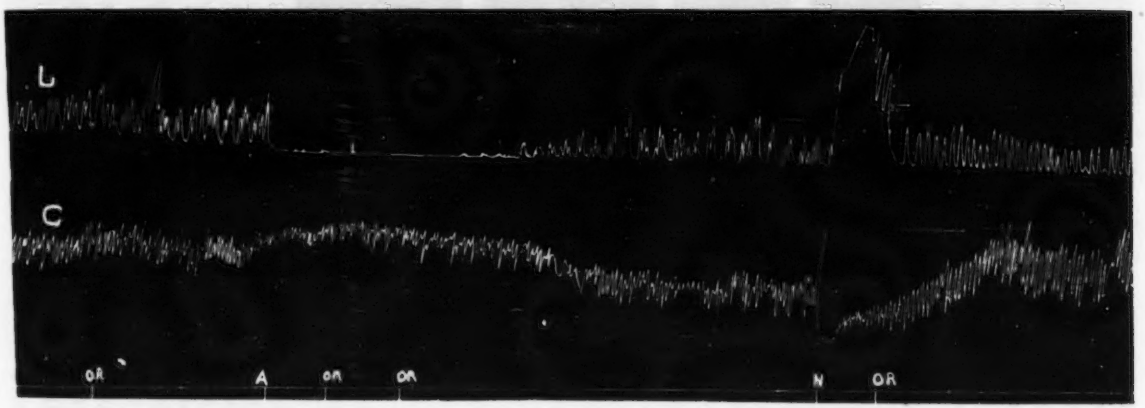

Fig. 4. Frog esophagus. Simultaneous records of circular system, *C*, and longitudinal system, *L*. *OR*, washed with oxygenated Ringer's solution. *A*, adrenalin (1:100,000) showing stimulation of circular system and inhibition of longitudinal system. *N*, nicotine (1:1,000,000) showing inhibition of circular system and stimulation of longitudinal system.

period of time there may be the development of a vigorous rhythm at the higher tone level. Recovery from this reaction is usually slow and requires several changes with oxygenated Ringer's solution.

4. Nicotine. Nicotine in dilutions of 1:100,000 acts essentially the same for both circular and longitudinal systems although the predominating action is different. When nicotine is applied to a preparation there is a prompt inhibition of the tone. The inhibition is more marked in the circular system than in the longitudinal system and the latter, if the tone is low, may be missed entirely (fig. 4). Following this inhibition there is a stimulating phase of nicotine which is more pronounced in the longitudinal system than in the circular system (fig. 4). In the circular system the stimulation is more of a tonus change while in the longitudinal system it appears as a sharp contraction and a rapid recovery. The nicotine effects could be obtained several times on the same preparation.

5. Atropine. If atropine be administered in dilutions of 1:100,000 after pilocarpine there follows a decrease in tone and also inhibition of the contractions which may have been initiated by the latter drug. This inhibition is uaually permanent and occurs in both circular and longitudinal systems. As a rule atropine is without effect on the circular system when under stimulation of pilocarpine.

In the case of the longitudinal system atropine is followed by a slight temporary inhibition. This reaction occurs with marked regularity.

6. Histamine and pituitrin. The addition of histamine hydrochloride in dilutions of 1:100,000 to preparations of frog's esophagus is followed by a prompt and abrupt inhibition of both the tone and the contractions (fig. 2, *C*). There is prompt recovery on removing the drug from the bath. The action of histamine is the same on both the circular and longitudinal systems.

There is a striking similarity between the action of histamine and that of adrenalin on the longitudinal system. On the circular system histamine inhibition is more constant than the adrenalin inhibition. The histamine reaction like that of adrenalin occurs after atropine. There is no instance in this series in which histamine was followed by a stimulating effect. This seems to indicate that the histamine action on this organ is nervous rather than muscular, a view previously expressed by Luckhardt and Carlson (2) in their work with this drug on the salamander lung. This is not in accord with the generally accepted conception of histamine as a direct stimulant of smooth muscle.

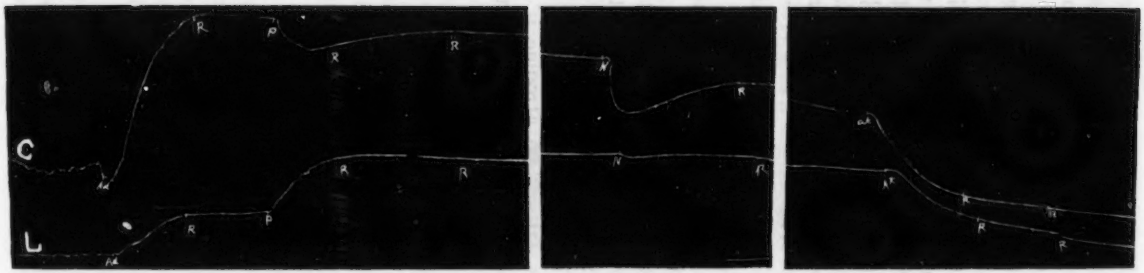

Fig. 5. Turtle esophagus. Stimultaneous record of circular system, *C*, and longitudinal system, *L*. *Ad*, adrenalin (1:200,000) showing greater stimulation on circular system than longitudinal system. *P*, pilocarpine (1:100,000) showing further stimulation of longitudinal system and only inhibition of circular system. *R*, wash with Ringer's solution. *N*, nicotine (1:100,000) showing inhibition of circular system and no action on longitudinal system. *At*, atropine (1:100,000) showing inhibition of both circular and longitudinal systems.

The frog's esophagus is described (3) as containing smooth muscle in both the longitudinal and circular systems. Addition of barium chloride to the bath had no effect on the esophagus.

Administration of pituitary liquid (Armour's) in dilution of 1:100,000 is followed by inhibition of both circular and longitudinal systems

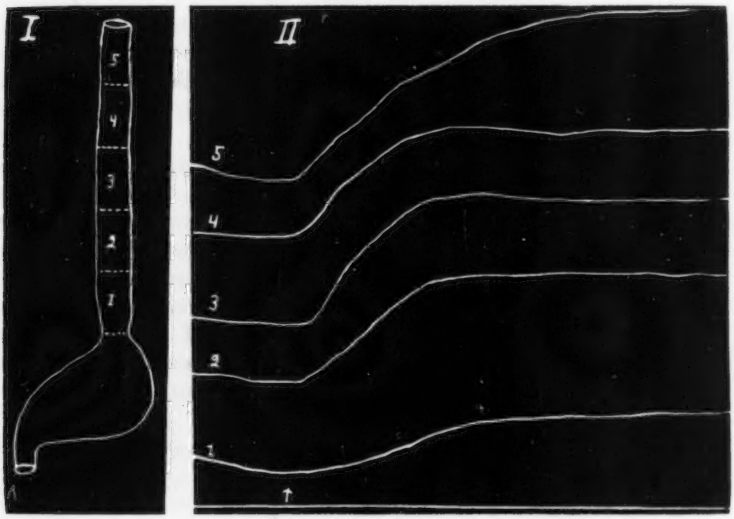

Fig. 6. Turtle esophagus. *I*, diagram of esophagus and stomach indicating relationships of rings used in this experiment. *II*, stimultaneous record of isolated rings of circular neuro-muscular system as indicated. Arrow indicates point of application of adrenalin (1:100,000) showing apparent decreased response of this system to adrenalin as region of cardia is approached.

(fig. 2, *D*). This confirms the work of Waddell (9) but it is not certain whether this effect is the result of the extract of the pituitary gland or of the histamine which this preparation is said to contain. This might account for the failure of this preparation as a specific myotropic drug.

RESULTS ON THE TURTLE'S ESOPHAGUS: *1. Adrenalin.* The action of adrenalin on the esophagus of the turtle is essentially the same for both longitudinal and circular systems. The addition of adrenalin in a dilution of 1:100,000 to preparations of turtle's esophagus is followed by a marked rise in tone (fig. 5, *Ad*). In many cases there is a distinct

initial inhibition of the tone preceding the stimulation. This effect does not appear in preparations with poor tone. The stimulating effect of adrenalin is more marked on the circular system than on the longitudinal system, and in both it continues for a long period of time in spite of repeated washings with adrenalin-free Ringer's solution. These reactions occur in preparations taken from all levels of the esophagus. In many preparations of the circular system there appears to be decrease in the response to adrenalin in those rings taken from the region of the cardia as compared with those taken from higher levels (fig. 6). All adrenalin reactions are obtained after atropine paralysis of the vagi.

2. Pilocarpine. The influence of pilocarpine in dilutions of 1:100,000 is the same for both circular and longitudinal systems. The drug causes a temporary inhibition of the tone, the degree of the inhibition depending on the amount of tone maintained by the preparation before the drug was added. If the circular system is in a condition of maximum contraction as the result of the administration of adrenalin, inhibition is the only effect produced by the administration of pilocarpine (fig. 5, *P*). In preparations with moderate tonus the initial inhibition is followed by a rise in tone (Fig. 5, *Lp*) and occasionally rhythmic contractions.

These pilocarpine reactions do not occur after atropine and are promptly antagonized by that drug (fig. 5, *At.*).

These results confirm the observations of Carlson and Luckhardt (1) who noted that stimulation of the vagi in turtles was followed by an inhibition of the tonus at least of the upper two-thirds of the esophagus. They also observed esophageal inhibition after pilocarpine injections. In their work they did not observe a motor effect from vagus stimulation or pilocarpine injections except in one case in which the animal was moribund and the esophagus in poor tone. This series appears to confirm the accuracy of their observation of that one case.

3. Nicotine. The addition of nicotine in dilution of 1:200,000 to the preparations of turtle's esophagus is followed by a profound inhibition of tone (fig. 5, *N*). This is more marked in the circular system and is dependent on the degree of tone maintained by the preparation. In many cases the inhibition is followed by an increase in tone.

4. Histamine. Histamine causes stimulation of the preparations of turtle's esophagus.

DISCUSSION OF RESULTS: *1. Dependence of the frog's neuro-muscular system on oxygen.* The neuro-muscular system of the frog's esophagus

is very sensitive to changes in its oxygen supply. This is especially true of the circular system when it is in its normal position inside of the longitudinal system. Turning the preparation inside out reduces this marked sensitivity. It gives the circular system more ready access to the oxygen in the bath. That this is an important factor is shown by the fact that it was necessary as a routine measure to turn all preparations inside out before any dependable reactions could be secured with the drugs. Preparations of the frog's esophagus will not develop spontaneous activity when atmospheric air is used instead of pure oxygen thereby indicating the dependence of this system on oxygen.

This is in marked contrast to the resistance to anemia of the nerve cells in the mesenteric plexus in cats, as pointed out by Cannon and Burket (4). These investigators showed that complete anemia of parts of the alimentary canal produced by compression between glass plates may last as long as three hours without loss of normal activity or of nerve cells from the compressed area.

The dependence on oxygen of the neuro-muscular system of the frog's esophagus is striking in its similarity to the dependence on oxygen supply of the centers for spinal reflexes as pointed out by Sherrington (5).

The gradual development of contractions in the isolated preparations indicates a period of recovery from the shock to the isolated peripheral nervous mechanism of making the isolated preparation. A similar condition was pointed out by Alvarez (6) who showed that strips taken from the cardiac regions of the frog's stomach suffer most from the trauma of excision and recover slower as compared to strips from the antrum. In the turtle this shock seems to be more profound. This apparent shock effect is also strikingly similar to that experienced in the spinal reflex centers.

In a previous report (7) attention was called to the fact that repeated stimulation of the vagi in turtles was followed by a rapid failure of gastric contractions in response to that stimulation. This seemed to indicate either a rapid fatigue or the development of some sort of a refractory condition within the vago-gastric motor mechanism. Both of these effects are similar to the corresponding points in Sherrington's analysis of spinal reflexes in mammals. In a later report (8) attention was called to the complexity of the peripheral neuro-muscular system in the turtle's stomach.

It would seem, therefore, that in these preparations we are dealing with an isolated nervous center which is sensitive to oxygen want and also to shock effects and that this center is controlling the spontaneous activities of the isolated neuro-muscular system of the frog's esophagus.

2. Nature of the preliminary inhibition. It was noted above in the case of nicotine on the frog's esophagus and at times on the turtle's esophagus that there was a primary inhibition which preceded the stimulating effects. This also occurred in the case of adrenalin and pilocarpine in the turtle.

The drug actions are all essentially the same on both systems. Both systems are present and potent. The delicate lever records primarily one neuro-muscular system but not to the complete exclusion of the other. It would seem therefore that the effect recorded in any case would be the algebraic sum of the activities of both neuro-muscular systems. This might be responsible for at least a portion of the primary inhibition recorded.

On the other hand it appears that we are dealing with a complex isolated nervous center capable of both inhibitory and motor activities. Assuming, at least for the present, that this is true and that the center is in a condition of tonic activity *the primary reaction of either system to the drug will depend at least in part on the relative tonus of the two systems.*

The circular and the longitudinal systems are, at least in part, mutually antagonistic, so that according to the law of reciprocal innervation, tonus or activity of the one should lead to depression of the other. Furthermore, the primary response of either system to drugs may be reversed by a reversal of the tonic state at the time of application of the drug.

Finally, it may be a case of double innervation of the musculature, the inhibitory innervation having a shorter latent period than the motor innervation. In that event both reactions would occur in a definite time relationship to each other which is never reversed.

3. Apparent failure of the so-called specific myotropic drugs to stimulate the smooth muscle of the frog's esophagus. It is noticed in the case of histamine, pituitary liquid and barium chloride that the usual stimulating effects were not obtained. There is also a striking similarity in the action of histamine on the frog's esophagus to that of adrenalin. A glance at table 1 will reveal a difference in the action of histamine on the frog's esophagus and on the turtle's esophagus. In the latter

animal the action is similar to that which is usually expected from histamine. As pointed out by Carlson and Luckhardt (1) "the conditions found in one animal group or species do not necessarily apply

TABLE 1

The action of drugs on preparations of the esophagus. Stimulation = +; inhibition = −; when both inhibition and stimulation were obtained in the same preparation, the primary action is placed above

	FROG ESOPHAGUS		TURTLE ESOPHAGUS	
	Circular system	Longitudinal system	Circular system	Longitudinal system
Non-aerated Ringer's solution	−			
Aerated Ringer's solution	−			
Oxygenated Ringer's solution	+*			
Adrenalin	+ (14 cases) − (7 cases)	−	− ++ all levels	− ++
Pilocarpine	+	+	− ++	++ −(after adrenalin)
Nicotine	− ++	− ++		
Atropine	−(after pilocarpine)	−(after pilocarpine) 0 −(temporary)		
Histamine	−	−	+	+
Pituitrin	−	−		

* The + in this case means that spontaneous activity developed.

to another group or species as the degree of differentiation in the motor control from the primitive condition appears to vary greatly in different animals."

4. Results on the intact animal as compared with the isolated preparations. It is evident that if the activities of the esophagus with its extrinsic innervation sectioned is dependent on a peripheral center the preparation will be more physiological if left in the animal with its circulation intact and without the traumatism necessary to isolate a strip or ring. In spite of the abnormal conditions surrounding the isolated preparation there is a striking confirmation of the results of Carlson and Luckhardt (1) on the physiological animal. The main difference seems to be in the magnitude of the reactions rather than their character and this may be easily explained on the basis of the difference in the recording systems.

SUMMARY AND CONCLUSIONS

Frog's esophagus. 1. Tonus and spontaneous contractions are maintained only in well oxygenated Ringer's solution. Atmospheric air cannot be substituted for pure oxygen. The circular system is more sensitive to lack of oxygen than to longitudinal system when in its normal position. Turning the esophagus inside out permits better aeration and more ready access of drugs to the circular system.

2. Adrenalin causes a uniform inhibition of the longitudinal system and stimulation (66 per cent of experiments) or inhibition (33 per cent of experiments) of the circular system. The adrenalin action seems to be more marked after atropine.

3. Pilocarpine stimulates both circular and longitudinal systems. Atropin counteracts this effect of pilocarpine.

4. Nicotine causes a primary inhibition followed by stimulation of the circular and longitudinal systems.

5. Histamine and pituitary liquid (Armour's) cause uniform inhibition of the circular and the longitudinal systems.

Turtle's esophagus. 1. The longitudinal and circular systems of the turtle's esophagus at all levels react the same to the drugs investigated.

2. Administration of adrenalin causes a profound and lasting stimulation usually preceded by a temporary inhibition. Atropine has no effect on this adrenalin action.

3. Pilocarpine causes a temporary inhibition followed by a marked stimulation. Only inhibitory effects occur in the circular system in preparations in a condition of maximum contraction following the administration of adrenalin. The preliminary inhibition may be absent if the tone is low.

4. Nicotine causes a profound inhibition. Following this in some cases there is an increased tone and then gradual recovery to the initial level.

5. Histamine is followed by an increase in tone.

The author wishes to take this opportunity to express his deep appreciation to Dr. A. J. Carlson and Dr. A. B. Luckhardt for their inspiration, for invaluable suggestions and unstinting help throughout the entire progress of this and previous studies in this series.

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THE WATER CONTENT OF THE TISSUES IN EXPERIMENTAL BERIBERI

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The object of this investigation was to ascertain whether there is an increase in the water content of the tissues in experimental beriberi, because of its bearing on edema as a phenomenon in starvation, protein deficiency (1) and other forms of maladies due to faulty diets. Our working hypothesis, as outlined by Doctor Carlson, was to the effect that incipient edema may be a consequence of all dietary deficiencies (quantitative and qualitative), macroscopically recognized edema appearing as a final symptom in a certain percentage of the individuals; but if the water content of the tissues is determined quantitatively, incipient edema may be universal. The work was started on beriberi, because the literature recognizes two types of this dietary disease, at least in man, the wet beriberi (edema) of infants, and the dry beriberi or polyneuritis of adults.

LITERATURE. Hirsch's Handbook in 1885 (2) mentions edema in legs, serous cavities and in lungs in some clinical cases of beriberi. Holst (3) occasionally found moderate edema under skin of legs and feet of polyneuritic pigeons. Holst and Frölich (4) noted frequent subcutaneous edema in scurvy guinea-pigs also. They call attention to a resemblance between abortive scurvy of guinea-pigs and ship beriberi (of man). Vedder and Clark (5) found little evidence of edema in polyneuritic chickens, except in cases of severe prostration, and then only a slight edema in pericardium at the base of the heart. Matsouka (6) finds that a congestion edema of the lungs is a clinical characteristic of beriberi. This goes hand in hand with a beginning dilatation and hypertrophy of the right heart. He also mentions presence of hydrothorax. Gibson and Concepcion (7) consider that hypertrophy of the heart and edema are typical of infantile beriberi, and mention signs of edema in beriberi dogs and pigs. Darling (8) observed edema of lungs in scurvy in Africa. Hess (9) spoke of subcutaneous edema in infantile scurvy, a widespread edema that does not pit on pressure, as it

infiltrates layers of integument and muscle. He noted a close relationship between this and infantile beriberi. McCarrison (10) in a study of the effect of deficiency diet on organs, finds in some of the pigeons such symptoms as these: excess fluid in the pericardial sac, band of edema at auriculo-ventricular juncture, edema in the lungs, some congestion in the lower pole of the left kidney, and edema in the groin. Effusions into serous sacs, although found frequently at autopsy, were rarely recognized clinically. He finds these symptoms in pigeons, not only after a diet of polished rice, but after a period of starvation. He also observes acidosis when antineuritic vitamine is absent. After diet of polished rice McCarrison (11) reports that the intestines of pigeons showed atrophy and congestion especially in the upper part, and occasionally he could see an edematous infiltration of the coats of the bowel. In scurvy guinea-pigs there was chiefly a hemorrhagic infiltration. He notes a relation between the form of beriberi occurring, and condition of suprarenals (12). Kellaway (13) confirmed McCarrison's findings. Bigland (14) in relation to edema, emphasizes effect of a food deficiency on certain endocrine organs. Rommel and Vedder (15) mention serous fluid in the pericardial sac and in the thoracic and abdominal cavities, and edematous lungs, occurring in wet beriberi in the pig. Williams and Saleeby (16) mention edema as a clinical symptom appearing frequently in beriberi, especially in infants. All these references to the presence of edema in beriberi and scurvy are based on macroscopic examination, except in a few cases.

EXPERIMENTAL METHODS. Beriberi was induced in the chickens and pigeons by feeding polished rice exclusively. Sand and water were also provided. The cages were large, and two to four birds were kept in a cage. Temperatures were taken before and after appearance of disease, and the typical fall in temperature occurred in every case. Just before the last stage of the disease, the animals were killed by decapitation and allowed to bleed dry. They were then opened up, and the organs dissected out and put in crucibles (previously dried and weighed). The crucibles and organs were weighed, heated in an oven (120°C.) till the weight became constant and the percentage of moisture computed. The same procedure was followed with controls. Animals used were over one year old, so the age factor was eliminated. The diet of the controls was unpolished rice. The organs studied were the brain, lungs, heart, liver, spleen, testes, kidneys, pancreas, stomach, intestines, muscle and skin. The brain was removed from its meninges, and cut off below medulla and excluding hypophysis. The lungs were

removed from the pleural sac. The heart was taken out of the pericardial sac, opened up and blood clots removed. Stomach and intestines were slit open, contents washed out, and adhering moisture removed by placing between two sheets of drying paper (in some series all of the intestine was used; and in others segments only). Muscles were taken from back of leg. Skin covering the leg was used. With chicks the gall bladder was dissected from the liver. In all cases masses of fat clinging to organs were removed, as the water content varies somewhat with lipoids. (17).

In the rats beriberi was induced by feeding a synthetic diet lacking in water-soluble B. The diet used was based upon that of McCollum and Davis (18), but with increased percentage of protein and butter fat to cover those factors. It was:

	per cent
Butter fat	12.0
Casein	18.0
Polished rice	66.5
Salt mixture	3.5
Agar-agar	about 1 per cent added for bulk

Salt mixture used was the one found satisfactory by McCollum and Davis, namely,

	grams
NaCl	5.0
K_2HPO_4	12.10
$CaH_4(PO_4)_2$	2.5
Ca lactate	29.5
Fe citrate	1.0

The rice (with agar-agar) was boiled in tap water till kernels were fairly soft, then salt mixture, casein, and butter fat added. The rats were given this mixture ad libitum, in addition to fresh water. Controls were given a normal diet of milk, oats, bread, carrots and greens.

Guinea-pigs were used for both beriberi and scurvy experiments. Beriberi was induced on the synthetic diet fed to the rats, but as guinea-pigs are reported (in the literature) to develop scurvy on a diet which causes beriberi in birds, they were given daily 5 cc. of orange juice extracted with Fuller's earth according to method of Hardin and Zilva (19). This is supposed to remove water-soluble B and leave the antiscorbutic factor. To induce scurvy in the guinea-pigs, bran and hay were fed ad libitum. In all the guinea-pigs, except four, the same method of killing and determining water content of organs

was used as in case of the chickens and pigeons. Four of the guinea-pigs and all of the rats were dried whole, after being killed with ether. In the rats the sciatic nerves were removed, and stained for degeneration by the Marchi method.

RESULTS. *Chickens.* Two sets of chickens were used: six chickens in the first set, and five in the second. (One chicken in each set did not develop beriberi during the duration of the experiment.) The first set was started on October 21, 1920. Losses in weight of the beribery chickens were as follows: 1175 grams to 1055 grams; 1365 grams to 1035 grams; and 1635 grams to 1110 grams. The birds gained weight for the first week or two of the feeding. Beriberi chickens were killed on November 29, 19 and 24, respectively. Two were in well-advanced stages, and a third in the last stage of the disease. The second set was started on December 24, 1920. Losses in weight were: 1935 to 1230 grams; and 1935 grams to 1570 grams. These also gained in weight for the first week or ten days. These beriberi chickens were killed on January 25 and 26, respectively. In no case was edema noted at post mortem.

The average water contents of the beriberi chickens and controls are given in table 1.

These results show a tendency toward increased water content in the skin, but the results are not constant enough to warrant a definite conclusion. No increase is seen in the other organs. In fact in some cases the total loss in water content more than balances the total gain.

Pigeons. Beriberi was induced in two sets of pigeons; six in the first and five in the second. The first set was started on October 21, 1920. Losses in weight of the beriberi pigeons were: 347 grams to 245 grams; 285 grams to 140 grams; 275 grams to 181 grams; and 360 grams to 198 grams. They gained in weight for the first week or ten days. The beriberi birds were killed on November 10, 22, 23 and 27, respectively. The second set was started November 19, 1920. Losses of weight of beriberi pigeons in this set were 320 grams to 210 grams; 315 grams to 250 grams; and 275 grams to 188 grams. They gained in weight for two to three weeks. These beriberi birds were killed on December 8 and January 3 and all were in fairly advanced stages. No signs of edema was observed at autopsy. The average water contents of the organs are given in table 2.

A study of table 2 shows in beriberi pigeons a distinct increase of water content in certain organs, and a somewhat slight but definite tendency toward it in other organs. The heart and intestines show the greatest

and most invariable increase. A rather constant tendency toward increased water content is seen in the lungs, skin, muscles and kidneys. The testes show a significant decrease in water content.

TABLE 1

Water content

		CONTROL CHICKENS			BERIBERI CHICKENS			H_2O + OR −
		High	Low	Average	High	Low	Average	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	Brain	79.50	79.00	79.25	79.58	77.98	78.90	− 0.68
	Lung	80.50	80.30	80.40	75.75	72.10	73.95	− 1.45
	Heart	74.50	70.60	72.55	72.30	64.58	69.36	− 3.19
	Muscle	78.90	75.50	77.20	76.50	72.50	75.13	− 2.07
	Skin	56.01	50.30	53.15	60.20	55.10	57.68	+ 4.53
	Stomach	74.00	72.40	73.20	71.50	68.30	69.63	− 3.57
	Spleen	84.12	76.73	80.43	75.49	74.90	75.19	− 5.24
	Pancreas	71.80	67.50	69.65	71.28	69.08	70.32	+ 0.67
	Intestines	79.90	77.00	78.45	77.50	75.17	76.50	− 1.95
	Kidney	76.65	76.55	76.60	77.80	74.70	76.66	+ 0.06
	Testes			83.40	79.60	75.0	77.37	− 6.03
	Liver	75.25	71.60	73.43	72.20	69.28	70.59	− 2.84
	Adrenals	76.60	69.77	73.19			59.00	−14.19
II	Brain	79.50	61.82	70.66	81.00	80.30	80.70	+10.04*
	Lung	78.10	76.40	77.30	77.10	77.01	77.05	− 0.25
	Heart	76.85	75.80	76.33	83.05	73.0	78.0	+ 1.67
	Muscle	76.60	75.50	76.05	75.60	74.20	74.90	− 1.15
	Skin	59.90	58.75	59.33	68.95	68.60	68.78	+ 9.45
	Stomach	75.70	73.50	74.60	74.45	72.64	73.55	− 1.05
	Spleen	77.75	77.25	77.50	78.20	76.55	77.45	− 0.05
	Pancreas	71.90	71.50	71.70	69.50	50.90	60.20	−11.50
	Intestines	77.25	75.95	76.60	78.90	78.40	78.65	+ 2.05
	Kidney	79.75	77.49	78.62	81.48	79.85	80.66	+ 2.04
	Testes	85.50	84.50	85.0	80.0	79.0	79.50	− 5.50
	Liver	74.10	69.90	72.0	74.75	71.25	72.45	+ 0.45
	Adrenals			67.0			73.0	+ 6.00

* Positive values not significant as one result far below all other values of brain.

Series I: 2 control chickens; 3 beriberi chickens.

Series II: 2 control chickens; 2 beriberi chickens.

Another set of pigeons was *starved* till loss in weight was equal to the average loss in the beriberi birds, to see if partial starvation rather than

the beriberi condition is the causal factor. The results are given in table 3.

It is evident from table 3 that starvation leads to an increased water content in the organs comparable to that in beriberi. This may be due to the acidosis of inanition.

TABLE 2

Water content

		CONTROL PIGEONS			BERIBERI PIGEONS			H_2O + OR −
		High	Low	Average	High	Low	Average	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	Brain	80.07	74.00	77.03	83.30	79.80	81.02	+ 3.99
	Lung	76.99	76.70	76.84	83.45	79.28	82.92	+ 6.08
	Heart	72.30	71.75	72.03	81.30	78.05	79.23	+ 7.20
	Muscle	75.15	72.30	73.73	77.30	74.40	75.80	+ 2.07
	Skin	41.17	31.10	38.64	69.85	64.55	66.10	+27.46
	Stomach	70.15	67.55	68.85	72.10	70.80	71.30	+ 2.45
	Spleen	75.75	75.48	75.61	84.60	62.50	72.71	− 2.90
	Pancreas	70.85	69.70	70.28	71.60	69.25	70.31	+ 0.03
	Intestines	74.58	65.50	70.04	84.40	81.25	82.48	+ 8.44
	Kidney	74.20	73.15	73.68	80.15	77.30	78.63	+ 4.95
	Testes			76.50			61.58	−15.02
	Liver	71.48	71.40	71.44	76.18	72.0	74.17	+ 2.73
II	Brain	80.80	80.0	80.40	80.25	79.44	79.86	− 0.54
	Lung	77.25	77.15	77.20	82.0	78.40	79.70	+ 2.50
	Heart	74.95	74.55	74.75	81.49	78.80	80.56	+ 5.81
	Muscle	73.60	72.60	73.10	76.90	75.0	76.18	+ 3.08
	Skin	45.42	14.77	30.10	65.75	61.55	62.97	+32.87
	Stomach	70.65	69.38	70.02	72.10	71.05	71.57	+ 1.55
	Spleen	78.60	70.95	74.78	71.68	67.90	70.06	− 4.72
	Pancreas	71.60	71.60	71.60	73.28	71.45	72.09	+ 0.49
	Intestines	72.60	68.15	70.33	81.55	78.00	79.98	+ 9.65
	Kidney	77.45	75.20	76.33	77.90	78.12	77.97	+ 1.64
	Testes	85.10	71.65	78.38	69.40	60.0	64.70	−13.68
	Liver	76.75	72.58	74.67	75.10	72.25	74.01	− 0.66

Series I: 2 control pigeons; 4 beriberi pigeons.
Series II: 2 control pigeons; 3 beriberi pigeons.

Guinea-pigs. Only two guinea-pigs were used in our attempt to produce beriberi, as it is not certain that the disease can be experimentally induced in guinea-pigs. We found no reference in the litera-

ture to beriberi in this species. One guinea-pig was used as a control. One of the experimental animals was lost by not being familiar with the symptoms of beriberi in the guinea-pig. The diagnosis of beriberi was checked by staining the sciatic nerves for degeneration (Marchi method). The symptoms observed in the guinea-pigs on the above diet were as follows: Gain in weight (equal to that of control) for three to four weeks; and then quick loss in weight and death in four to five days. Both guinea-pigs showed roughness of hair after four weeks

TABLE 3

Water content

	TWO CONTROL PIGEONS			FOUR STARVED PIGEONS			H_2O + OR −
	High	Low	Average	High	Low	Average	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	79.85	79.49	79.67	81.00	79.80	80.54	+ 0.87
Lung	77.45	76.75	77.22	82.90	74.45	79.32	+ 2.10
Heart	75.05	74.10	74.60	79.80	77.55	78.80	+ 4.20
Muscle	75.65	74.49	75.07	75.30	75.40	77.50	+ 2.43
Skin	62.90	61.60	62.25	70.70	60.50	65.93	+ 3.68
Stomach	71.75	69.10	70.43	70.75	68.05	69.49	− 0.94
Spleen	81.35	73.35	77.35	66.70	57.50	61.80	−15.55
Pancreas	72.60	71.73	72.17	75.50	71.51	73.44	+ 1.27
Intestines	70.95	70.70	70.83	78.30	74.95	76.44	+ 5.61
Kidney	77.01	74.70	75.86	81.10	76.55	78.80	+ 2.96
Testes			85.98	79.50	68.45	73.98	−12.0
Liver	68.80	68.50	68.65	74.48	71.52	72.94	+ 4.29*

* Positive values not significant when water content of the controls is compared with controls of table 2.

on the diet. From this on the animals remained in about the same condition and maintained a good appetite, till appearance of definite symptoms of the disease. But the animals became gradually more noisy when they were being fed. In the guinea-pig used, definite symptoms appeared at the end of the sixth week. The animal became very weak, and was uncertain in his movements (stupid) with subnormal temperature, and absence of the usual nervous shivering on being handled. No signs of scurvy were seen on autopsy, showing that the extracted orange juice had protected him from scurvy. Results are given in table 4.

Table 4 reveals no increased water content in any organ.

TABLE 4

Water content

	ONE CONTROL GUINEA-PIG	ONE BERIBERI GUINEA-PIG	H_2O + OR −
	per cent	*per cent*	*per cent*
Brain	80.90	80.60	−0.30
Lung	80.60	81.10	+0.50
Heart	79.35	79.43	+0.08
Muscle	77.75	76.10	−1.15
Skin	70.50	63.05	−7.45
Stomach	79.0	80.80	+1.80
Spleen	79.10	78.60	−0.50
Intestine	87.30	85.30	−2.0
Kidney	78.85	79.40	+0.55
Liver	74.35	76.60	+2.25

Scurvy was developed in two series of guinea-pigs. In the first series six animals were used. They were killed after being on the special diet for two to three weeks. The animals lost weight, first slowly, then more rapidly. Other external manifestations were roughness of hair, sitting crouched up, and in some cases, squealing when handled. Diagnosis of scurvy was confirmed by typical appearance on autopsy. The results on this first series are given in table 5.

TABLE 5

Water content

	THREE CONTROL GUINEA-PIGS			THREE SCURVY GUINEA-PIGS			H_2O + OR −
	High	Low	Average	High	Low	Average	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	81.10	71.98	80.46	80.90	80.15	80.75	+0.29
Lung	81.49	51.60*	71.15	80.60	79.90	80.30	+9.15
Heart	81.30	80.10	80.00	80.70	79.20	80.20	−0.60
Muscle	77.95	77.20	77.53	76.95	76.06	76.51	−1.02
Skin	69.95	67.50	68.72	71.60	64.03	67.57	−1.15
Stomach	81.40	80.10	80.90	82.0	80.50	81.50	+0.60
Spleen	79.75	78.08	79.74	79.45	74.05	77.27	−1.47
Intestines	85.70	82.90	84.50	65.60	84.45	84.88	+0.38
Kidney	84.55	77.80	80.58	81.55	80.75	81.08	+0.50
Liver	76.60	74.60	75.73	74.90	70.75	73.24	−2.49
Adrenals	64.45	57.00	61.03	75.50	60.90	67.20	+6.17

* This result probably not correct as it is so much lower than the rest.

Table 5 reveals no definite edema in any organ in the scurvy guinea-pigs.

In our method of studying the separate organs, the serous sacs and cavities are not included in the determinations, hence there might be some edema that our method fails to disclose. In order to test this

TABLE 6

Water content of entire body

TWO CONTROL GUINEA-PIGS	TWO SCURVY GUINEA-PIGS	INCREASE IN H_2O
per cent	*per cent*	
74.17	77.60	+3.43
73.63	76.70	+3.07

possibility, scurvy was produced in two additional guinea-pigs, and two animals used as controls. The animals were killed by ether and desiccated in toto (including the blood). The results are given in table 6.

Rats on beriberi diet. Eight rats were employed in this test, two serving as controls. They weighed between 50 to 80 grams at the beginning of the experiment, and were nearly of the same age, but as all were over five weeks old, age would be eliminated as a factor (20). After nine weeks on the deficient diet, they were killed with ether and dried

TABLE 7

Water content of entire body

TWO CONTROL RATS	SIX BERIBERI RATS	H_2O + OR −
per cent	*per cent*	*per cent*
70.07	69.50	−0.29
69.50	69.25	−0.54
	71.25	+1.46
	72.53	+2.74
	72.40	+2.61
	73.0	+3.21

whole as described above. The rats did not gain as well as the controls, but otherwise showed no external symptoms. There was some degeneration of the sciatic nerve in every animal, showing the lack of water-soluble B had produced the nervous lesions of polyneuritis. The results are shown in table 7.

In four of the rats there is a small but definite increase in water content. The remaining two are practically identical with the controls.

CONCLUSIONS

1. There is no definite increase in water content of the organs of beriberi chickens, except possibly in the skin.

2. In beriberi pigeons there is an increased water content in the intestines, and in the heart, the lungs, the skin, the muscles and the kidneys. Starvation, in proportion to the loss in weight in the beriberi birds, leads to a similar increase of water in these tissues.

3. In one beriberi guinea-pig, no increased water content was found in the organs studied.

4. In scurvy guinea-pigs, increased water content was not detected in determinations of separate organs, but when the animals were dried whole, such a tendency was noted.

5. In beriberi rats (entire body), there was increased water content in some cases and in others there was no change.

6. Quantitative analysis (separate organs, or the entire animal) appears to reveal increased water content of tissues (or incipient edema) in dietary deficiency diseases, where edema is not in evidence on the usual gross examination.

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STUDIES IN CARBON MONOXIDE ASPHYXIA

II. The Growth of Neuroblast in the Presence of Carbon Monoxide

A demonstration that this gas has no direct toxic action upon nervous tissue

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In severe carbon monoxide asphyxia the chief damage is usually wrought upon the central nervous system. This apparently selective action of the gas led to the belief that carbon monoxide exerts a specific toxic action upon nervous tissue. From the clinical and pathological sides the acceptance of this specificity affords an easy explanation of an otherwise difficult problem. Physiological evidence indicates, however, that aside from its displacement of oxygen from the blood, carbon monoxide is as innocuous as nitrogen (1). The nervous sequelae of the poisoning follow as one of a train of profound physiological alterations, due primarily to the anoxemia induced by the combination of carbon monoxide with hemoglobin and exclusion of oxygen.

In the present work an attempt has been made to obtain some direct evidence upon this problem by observing the action of carbon monoxide upon cultures of chick neuroblasts growing in vitro. The nerve cells under these conditions maintain their gaseous exchange directly through the plasma, in which they are suspended, without the intervention of hemoglobin. By thus dispensing with hemoglobin, the asphyxiant action of the carbon monoxide is avoided. The concentration of the gas in the medium about the cells is determined solely by the tension of gas in the atmosphere with which the plasma is in contact. For this reason it is possible to expose the neuroblasts to far greater amounts of carbon monoxide than would be possible in the body.

As will be seen in the following experimental work carbon monoxide even in concentrations as high as 79 per cent acts as an inert gas toward

nerve cells. When the carbon monoxide is supplied in the form of illuminating gas, i.e., fattened water gas, this is not the case. Some accessory substance in this gas has, at least under in vitro conditions, a highly toxic action upon growing nerve cells.

Experimental methods. The general technique of preparation and the normal appearance of cultures of chick nervous tissue grown in vitro have been fully described by R. G. Harrison (2) and his pupils (3). I take this opportunity to express my indebtedness to Professor Harrison for his personal advice and suggestions during the course of this work. In brief the technique is as follows: Fertile hen's eggs are incubated at 39°C. for 48 hours or longer. One of these eggs is then placed upon its side, and the upper surface sterilized by washing with alcohol and dried.

TABLE 1

Showing the reaction of chick nervous tissue in the presence of air, carbon monoxide, and air with 0.1 per cent illuminating gas

ATMOSPHERE TO WHICH NERVOUS TISSUE WAS EXPOSED	NUMBER OF CULTURES	NUMBER OF GROWTHS	PER CENT GROWTH	REMARKS
Atmospheric air	36	31	86.1	Growth normal in appearance
79 per cent carbon monoxide and 21 per cent oxygen	20	18	90.1	Growth normal in appearance
Atmospheric air and 0.1 per cent illuminating gas	18	0	0	Tissue dead and partially degenerated

An area of the shell about 3 cm. in diameter is cut away with scissors, thus exposing the chick embryo. Under strong illumination and a binocular microscope small segments of neuroblastic tissue are cut away with sharp-pointed iridectomy scissors. These fragments are floated into warm saline and cut into smaller bits. One of these is then drawn into a capillary pipette and placed in a drop of chicken plasma resting upon a microscope cover slip. Asepsis is maintained throughout. The plasma employed as the supporting medium for the tissue is obtained by centrifuging cooled chicken blood; the blood is drawn by means of a Luer syringe and needle from the superficial vein upon the medial aspect of a fowl's wing.

The cover slip with its drop of plasma containing the tissue is inverted over a glass ring the surfaces of which are coated with petrolatum.

This in turn rests upon a microscope slide. Thus sealed, the hanging drop culture is aerated only by the atmosphere within the cell formed by the ring, the slide, and the cover glass. But in the present work the slide used was pierced with a 2 mm. central hole opening into this cell.

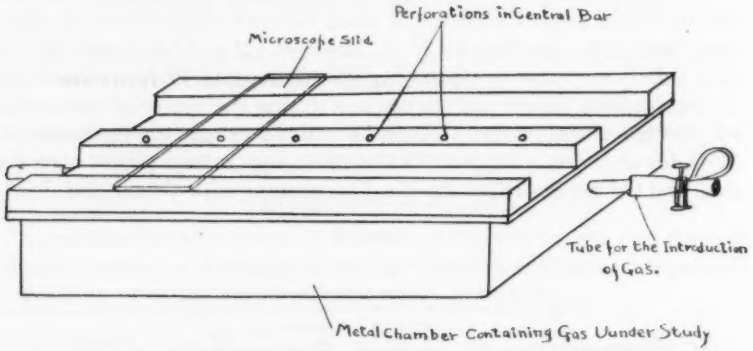
Perforations in Central Bar
Microscope Slide
Tube for the Introduction of Gas.
Metal Chamber Containing Gas Under Study

Fig. 1. Apparatus for exposing in vitro cultures to gases

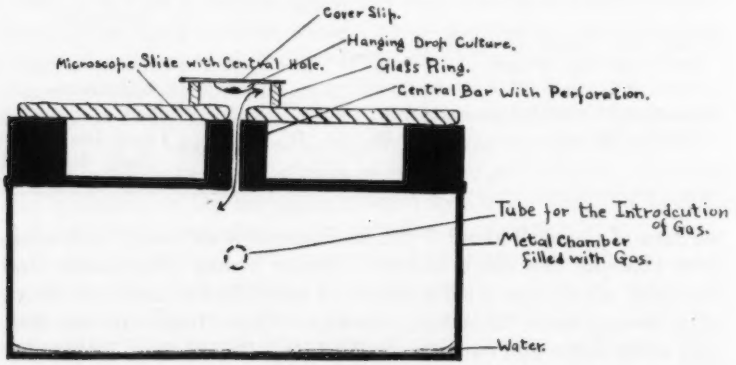

Fig. 2. Sectional view of apparatus showing culture in place

In order to study the effects of gases other than atmospheric air upon the growing nervous tissue it is necessary to employ some means of introducing and maintaining the gas within the cell. For this purpose the following apparatus was designed: It consists (figs. 1 and 2) of a metal chamber acting as a gas reservoir, which is surmounted by three metal bars placed equidistant and extending longitudinally. The

middle one of these bars is perforated at 3 cm. intervals with small openings which lead to the chamber below. The chamber is made of sheet brass and is 25 cm. long, 10 cm. wide and 4 cm. deep. The bars are of square brass, 1.5 cm. thick and extend the full length of the chamber. At either end of the metal chamber are small tubes fitted with short lengths of rubber tubing and pinch clamps.

The chamber is sealed by coating the bars lightly with petrolatum and placing microscope slides transversely across the three in such a manner as to cover the openings. The gas under study is introduced by connecting one of the tubes to a gasometer filled with the desired mixture. With both pinch clamps open, a sufficient volume is passed through to afford thorough flushing. The effluent tube is then connected to a gas analyzer and a sample drawn for analysis, after which both clamps are closed. The atmosphere in the chamber is kept moist by introducing a little water.

In order to allow diffusion of the gas from the chamber into the cell containing the suspended culture, the slide on which the culture is carried is pierced as already mentioned. The end of this slide is abutted against one of the plain slides used to seal the chamber and is pushed across the bars, thus replacing the blank slide, until the central perforation through the slide into the cell is over one of the openings of the central bar leading into the chamber. Free diffusion is thus established between the gas in the chamber and the atmosphere in the culture cell. The apparatus thus sealed and charged with gas is placed in an incubator at 39°C. The slight increase in pressure incident to the rise in temperature is relieved by momentarily opening one of the pinch clamps.

To remove the culture for examination the perforated slide is replaced by a blank slide in the same manner as described above. By this method the gas in the chamber is maintained without loss. This fact has been verified by analysis after each period of incubation.

In this study the gases used were: *a*, atmospheric air as control; *b*, 79 per cent carbon monoxide and 21 per cent oxygen; and *c*, 0.1 per cent illuminating gas in air. The carbon monoxide was prepared by the action of concentrated sulphuric acid upon formic acid at 80°C., and was washed repeatedly with caustic alkali solution and water to exclude possible extraneous irritant gases incident to its manufacture. The illuminating gas was the average run supplied to the City of New Haven. The local gas company has kindly furnished the following analysis: carbon dioxide, 3.5 per cent; benzol (C_6H_6), 1.0 per cent; olefines ($C_n H_{2n}$), 5.6 per cent; oxygen, 4.0 per cent; carbon monoxide, 21.4 per cent; hydrogen, 43.2 per cent; methane, 19.8 per cent; nitrogen, 5.1 per cent.

Examination was made of the tissue cultures after 24, 48 and in some cases 72 hours of exposure to the various atmospheres. In the accompanying table (page 245) are given the results obtained.

It is here to be seen that the percentages of successful growths in air (79 per cent nitrogen and 21 per cent oxygen) and in an atmosphere of the same oxygen tension but 79 per cent of carbon monoxide instead of nitrogen, were virtually the same. Indeed the latter, by the mere chance of experimentation, shows a slightly better percentage than the former—90.1 against 86.1. The growth observed with the atmosphere containing 79 per cent of carbon monoxide was in all respects as normal as that obtained when the gas in the cell was atmospheric air.

The fact that delicate neuroblast will propagate in an apparently normal manner when exposed to a tension of carbon monoxide so great as to be in the body physologically impossible, is conclusive evidence that carbon monoxide does not exert a specific toxic action upon nervous tissue.

Illuminating gas does, however, contain some substance toxic to nerve cells for no successful growths at all were obtained even in highly diluted gas (0.1 per cent). This is obviously due to some constituent of the gas other than carbon monoxide. But it is important to remember that the exposed tissue in a culture is probably more sensitive than the normal living animal or man. The fact that illuminating gas is more toxic than its content of carbon monoxide would wholly account for, has been noted previously in this laboratory (4), but one must not over-estimate this fact. It remains true that the toxicity of illuminating gas when inhaled is chiefly due to carbon monoxide; but in the body carbon monoxide acts through combination with hemoglobin and consequent asphyxia, and not specificially upon nerve tissue.

SUMMARY AND CONCLUSIONS

1. A technique is described whereby the growth of in vitro cultures of nervous tissue from the chick may be tested in any desired atmosphere or mixture of gases.

2. Carbon monoxide even in concentrations of 79 per cent is found to have no ill effect upon growing nerve cells. In this respect this gas is as neutral as nitrogen.

3. Illuminating gas has been studied in like manner and found to be toxic for neuroblast cultures in concentration of even as little as 0.1 per cent.

4. These observations indicate that carbon monoxide has no specific reaction with nerve tissue but acts in the body only through the asphyxia incident to its combination with hemoglobin.

5. These experiments indicate, also, that illuminating gas contains another toxic substance or substances; but this observation is not to be interpreted as weighing against the fact, well demonstrated, that asphyxia from inhalation of illuminating gas is chiefly due to carbon monoxide.

I wish to express my thanks to Prof. Yandell Henderson for advice and criticism.

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THE RELATION OF SPLENECTOMY TO GROWTH AND APPETITE IN THE RAT[1,2]

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Throughout the entire literature on splenectomy is repeatedly found the idea that increased appetite and decreased activity result from the operation. In Richet's (1) extensive experiments on the effects of splenectomy in dogs, he reported that after the operation the animals ate more than "normal" dogs did but gained slightly less in weight. When the splenectomized animals starved, they lost weight more rapidly than control animals under the same conditions and when they failed to continue to eat more than control dogs, they finally died of starvation. According to Richet, the splenectomized animals maintained a body temperature slightly higher than that of the controls and showed a very active metabolism. Danoff (2), moreover, concluded that although the respiratory quotient remained unchanged, there was an increase in carbon dioxide output and oxygen consumption after splenectomy. Richet used thirteen animals some of which he discarded for one reason or another when he drew his conclusions. The diet—a soup of bread, milk and water ad libitum and cooked horse meat fed separately—which was given by Richet, offers some difficulty in accurate measurement. The matter of normal and abnormal growth and the correlated food intake is one which must be subjected to statistical methods of study. Hitherto, in the case of the dog, these phenomena have not been measured in sufficiently numerous cases nor have the feeding conditions been carefully enough supervised to warrant drawing fine distinctions between normal and abnormal rates of growth. Moreover, unless dogs are reared in the laboratory on a definite feeding program, early habits, diversity

[1] Part of the expenses of the investigation was defrayed by a contribution from the Russell H. Chittenden Research Fund for Physiological Chemistry.

[2] This work was reported at the December (1921) meeting of the Society for Experimental Biology and Medicine, New York.

of previous treatment and variation in breed play a large part in their behavior under experimental conditions. Reports from the older literature that "the animals were increasingly voracious after splenectomy," etc., must be considered more as interesting observations than as scientific data. In the case of rats, Prym (3) stated that increased appetite was observed; but no data are available. Henn (4) likewise reported experiments on the growth of rats, kittens and puppies after splenectomy but the food intake of the animals was not carefully controlled.

It is desirable, therefore, to have more accurate information on the question of appetite and growth after splenectomy. The white rat is an animal admirably suited to experiments on food intake and growth, for these have been extensively studied and average curves for thousands of individuals are available. In view of the persistence of this idea of the relation of the spleen to appetite, we have studied the effect of splenectomy on the food intake and growth in the white rat.

The animals were splenectomized as nearly at the age of 40 days as possible. The simple operation was done under ether anesthesia and the splenic vessels were ligated *en bloc* with silk. Although the average growth and food intake curves of large numbers of individuals are available from other studies, it was thought desirable to run control animals under the same environmental conditions. In the control rats an abdominal incision was made and the spleen drawn out and replaced, the whole operation simulating as far as possible the splenectomy.

The rats were fed ad libitum upon the diet of purified materials described by Osborne and Mendel (5). The technic has been outlined by Ferry (6). Vitamin-B was provided by 0.4 gram dried brewery yeast daily, fed apart from the other food. The rats were weighed twice each week and their food intake was determined.

Of the first group of rats, five were observed for 34 weeks after splenectomy; three were observed for 43 weeks. All of these animals grew normally or above for about the first 140 days. In no case was this normal growth accompanied by consistently increased food intake. Usually our splenectomized rats ate slightly less food than indicated by the curves for the food intake of normal rats.

From about the 140th day on, a marked change occurred in growth rate of the male rats of both the experimental and the control groups. The increments in body weight were smaller than expected and continued so until, at the end of the experiment at the age of 240 days, the male rats were from 50 to 75 grams below the normal average weight for this age according to Osborne and Mendel (7). Rats 8 and 10, both

splenectomized males, were exceptions in that they continued growing at the normal rate for 210 days before they declined. In both these animals the food intake after the 200th day was slightly above that of our controls. Unfortunately, average normal food curves were unobtainable for this advanced age in rats.

Better growth rate was observed with the female rats. Three of the four splenectomized females grew at a rate above normal during their entire life. The fourth (rat 4) declined very slowly from the 160th day being, however, only 25 grams below the average normal weight at the 240th day. None of these splenectomized females ate appreciably more food than did the controls nor more than that indicated by the average food intake of normal animals on the same ration.

Rats 7 and 15, two of the splenectomized females, were mated with a splenecotmized male (rat 8) and raised litters. The young were apparently normal. Rat 15 raised seven of a litter of eight and rat 7 raised three of a litter of eight, the remainder being killed because of poor condition. Eight of these "second generation" rats were splenectomized and their growth and food consumption likewise observed for 160 days. Here again all of these animals grew at a rate equal to or slightly greater than that indicated by the normal average curves. The rate of food intake was equal to or very slightly less than what was expected.

From these observations on splenectomized rats and on their splenectomized progeny, *there is no indication whatever of an increased appetite as a result of the removal of the spleen.* The animals grew at the same rate on the same food intake as did the control rats, until the 140th day, as was indicated by the average normal curves for growth and food intake. The data obtained from these experiments seem to present conclusive evidence against the idea that the presence or absence of the spleen plays a part in affecting the nutritive requirements of the white rat. These results on the nutrition of the rat after splenectomy harmonize with those of Goldschmidt and Pearce (8) and of Paton (9) who studied the metabolism of the dog after splenectomy.

Among the many functions attributed to the spleen has been the one of altering the blood. In the older literature there are many conflicting results on the blood after splenectomy, anemia being reported in some cases and not in others. The extensive work of Pearce (10) and his associates on splenectomized dogs seems to point to a definite anemia of varying degrees of severity and of varying duration. Furthermore, the increased resistance of the erythrocytes of splenctomized animals

to hemolysis by hypotonic solutions has been reported repeatedly. On the other hand, Paton, Gulland and Fowler (11), working on dogs, cats and rabbits, found no effect on the blood picture following splenectomy. Wolferth (12) reported a slight transient anemia following the splenectomy in white rats.

Erythrocyte counts were made in five of our splenectomized rats whose parents had likewise been splenectomized. The end of the tail was clipped and the blood sample was taken when the blood oozed out freely. As will be seen from the table, counts were made before splenectomy, 4 to 7 days after splenectomy, a week later, and then every 4 days until 7 weeks after splenectomy. It was hoped thus to detect blood changes occurring within 2 weeks and especially those occurring from 3 to 6 weeks after splenectomy at which time, according to Pearce, the anemia in dogs reaches its maximum severity. The values for the red cell count are somewhat variable, but in no case was there an unmistakable anemia after removal of the spleen. There was a mere suggestion of a decrease in rats 25 and 23 but even in these animals the effect was transient and of small magnitude.

TABLE 1

RAT 19		RAT 20		RAT 23		RAT 24		RAT 25	
Days after splenectomy	Red count	Days after splenectomy	Red count	Days after splenectomy	Red count	Days after splenectomy	Red count	Days after splenectomy	Red count
	6,980,000		6,180,000		8,530,000		8,370,000		8,290,000
8	7,080,000	8	6,450,000	4	7,800,000	4	8,630,000	4	7,430,000
11	7,470,000	11	7,150,000	9	7,670,000	9	7,600,000	9	7,300,000
16	7,350,000	16	7,200,000	16	8,500,000	16	8,640,000	16	9,000,000
21	7,800,000	21	7,680,000	19	8,390,000	19	7,910,000	19	7,410,000
28	7,300,000	28	7,710,000	23	8,420,000	23	7,560,000	23	8,370,000
31		31	8,160,000	26	8,640,000	26		26	7,660,000
35	8,510,000	35	8,250,000	30	8,850,000	30	7,800,000	30	8,190,000
38	7,640,000	38	7,180,000	33	8,310,000	33	8,370,000	33	8,480,000
42	8,180,000	42	7,560,000	37	8,860,000	37	8,250,000	37	8,970,000
45	7,870,000	45	8,030,000	41	8,500,000	41	8,430,000	41	8,780,000
49	7,930,000	49	7,670,000	45	8,230,000	45	8,020,000	45	8,860,000
52	7,920,000	52	7,820,000	48	8,900,000	48	8,500,000	48	8,950,000
56	8,050,000	56	7,770,000						
59	8,050,000	59	7,830,000						

In experiments conducted in this laboratory, Geiling and Green (13) reported that, after hemorrhage, splenectomized rats regenerate their blood in normal time. These facts taken together with our observations make it appear doubtful that removal of the spleen in the white rat results in changes of appreciable magnitude in the erythrocyte count.

Rats 7, 10, and 15 were sent to Dr. Donaldson of The Wistar Institute who kindly examined them for any structural changes resulting from splenectomy. The organs and bones were normal in weight and measurement and showed no effect of splenectomy. The marrow of the bones of rats 7 and 10, animals which had been bled small amounts for blood counts and hemoglobin estimations for a short time after splenectomy, showed the expected change from yellow to red while the bones of rat 15 did not show this change.

SUMMARY

A study of the body weight and food intake of the white rat after splenectomy gave no evidence of an increase in appetite or variation from the normal rate of growth.

A study of the red cell count on five splenectomized rats which were the progeny of splenectomized parents showed no anemia followed the removal of the spleen.

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THE RELATION OF THE ADRENALS TO FATIGUE[1]

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Experimental work of recent years has indicated that the adrenal medulla is not essential to life (1). Operations in which all of the medulla of a single remaining adrenal has been destroyed have produced no specific symptoms.

Although it has been shown experimentally that epinephrin, the secretion of the medulla, can modify the activity of many tissues in the body, the question as to how much this takes place normally is unanswered.

Stewart and Rogoff (2) have been unable to show that a reduction of the epinephrin output to $\frac{1}{30}$ of the normal or less produces any difference in the animal. In view of the very suggestive emergency theory of Cannon (3) it would be interesting to know whether such animals as those just mentioned could meet conditions of stress as well as normal animals. So far as we know the animals of Stewart and Rogoff were permitted to lead a quiet life as most laboratory animals do.

We have tested the ability to work and to withstand fatigue, and in one instance the effect of pregnancy, in animals with a reduced epinephrin output.

We have obtained evidence that animals, with but one adrenal and that denervated, do not possess the endurance of normal animals. Our results with the denervated pupil suggest that this is due to an insufficient supply of epinephrin.

Care of animals. Many of our animals were observed for some time and were tested before the operation. In most instances the removal of one adrenal and denervation of the other took place at one operation, the abdominal path being used. Every possible precaution was taken to prevent infection, the regular hospital routine being followed. The animal was carefully protected by sterile cotton and an abdominal

[1] This study was aided by a grant from the Research Fund of the American Association for the Advancement of Science.

bandage in the form of a jacket with openings for the four feet. Primary infections were absent. If secondary infection occurred, which was uncommon, the experiment was discontinued except where the infection yielded readily to antiseptic treatment.

The animals were fed with boiled liver, milk or bread and milk, occasionally table scraps and yeast at intervals. Even kittens thrived upon this diet.

They were housed in a large, well-lighted room connecting with a large outdoor enclosure. They were permitted to roam at will through this room and enclosure.

Treadmill for fatigue. The apparatus for fatiguing animals consisted of a wooden treadmill with an inside diameter of 31 inches and an inside width of 11 inches. The treading surface was composed of slats placed far enough apart for ventilation. The sides were covered internally with transparent celluloid to keep the animal from clinging to the wire gauze. Access to the inside was by means of a door in the circumference. It was arranged so that at each revolution of the mill an electrical contact was made. A signal magnet, for a kymograph record, and an electrical counter were connected in the circuit. In some instances an electric fan was directed toward the tread in order to increase the ventilation.

The mill was turned by means of a crank so that the operator could vary the rate or change the direction to suit the whim of the animal or the condition of fatigue. It was found from experience that by carefully watching the animal and varying the speed to accommodate it and also changing the direction when it appeared tired of pacing, much more work could be obtained. With practice it is possible to induce many cats to work. which ordinarily refuse to work in the mill.

There are cats which never work satisfactorily in the treadmill either from clumsiness or obstinacy. This must be considered in the tests.

The treadmill method was found to be more satisfactory than leading an animal around the room because when the animal becomes slightly fatigued or is disinclined to work he permits himself to be dragged and does little work. In the treadmill the stimulus for work is greater and much more work is done in a unit of time than in walking on the level.

Observations. A period of ill health greater than that in controls sometimes follows removal of one adrenal and denervation of the other. This stage is characterized by loss of appetite, loss of weight, change in behavior and in some cases a lowered temperature. Diarrhea is common

but seems to be due to cutting the splanchnic nerve, for it is produced in operations where only the splanchnic nerve is cut. The following gives briefly a comparison of denervated adrenal animals and control animals.

CAT NUMBER	WEIGHT LOST	DAYS TO REGAIN WEIGHT FROM TIME OF OPERATION	LOWEST TEMPERATURE RECTAL
One adrenal removed, other denervated			
101	17% in 13 days	35 to regain all	Normal
103	29% in 16 days	28 to regain all	Normal
104	36% in 29 days	36 to regain 7% (accidently killed)	36.6° C. at 29 days normal at 37 days
105	21% in 12 days	60 to regain 5%	37° C. at 14 days normal at 19 days
111	14.3% in 15 days	41 to regain 11.6%	Normal
One semilunar ganglion removed			
102	6.0% in 9 days	12 to regain all	Normal
One semilunar ganglion removed opposite adrenal manipulated			
108	12.0% in 4 days	14 to regain all	Normal
One adrenal removed, adrenal vein tied on other side			
109	10% in 30 days	47 to regain all	Normal
Semilunar ganglion dissected free except from splanchnic and capped with rubber			
123	6.8% in 20 days		

Although it would require a large number of animals to reach a definite conclusion our animals from which one adrenal had been removed and in which the nerves to the other gland had been cut, certainly were affected more than the controls. The greater loss in weight, subnormal temperature and change in behavior might be accounted for by the decreased adrenal function, the recovery, by regeneration of the nerves and a consequent increase in function.

The ability of all animals to work and to withstand fatigue has been tested by the treadmill. In our later work we used the denervated eye as a possible test for epinephrin in the work tests. The following evidence indicates that this test can be used for epinephrin output in fatigue. This is based upon the most reasonable interpretation of our observations which follow.

The denervated eye reaction as a test for epinephrin in fatigue. If an animal, from which one of the superior cervical ganglia has been removed, is caused to work in a treadmill, after a few minutes the denervated pupil becomes larger than the control pupil. This might be due to inhibition of the tonic activity of the ciliary nerve or it might be due to the action of an increased output of epinephrin from the adrenals. Meltzer and Auer (20) have shown that the sensitivity of the pupil reaction to epinephrin is increased very much above normal by previous removal of the superior cervical ganglion.

In some animals the response of the denervated eye develops more easily than in others. We chose a cat in which there was always a good dilatation of the denervated pupil as compared to the control, within two or three minutes after beginning the work in the treadmill. This dilatation continued throughout the test. The right adrenal had been removed twenty-five days previously. The remaining adrenal was removed through as small an opening as possible by the lumbar path. On the morning following this operation, although the cat worked for one hour in the treadmill, travelling 728 m., no dilatation of the denervated pupil occurred throughout the experiment, this pupil being smaller than the control at all times as in a condition of rest. In the afternoon of the same day a further test of 430 m. failed to produce a positive pupil reaction. Yet previous to the removal of the second adrenal a good dilatation of the denervated pupil occurred invariably within two to three minutes and fifty or more meters. Apparently taking out the second adrenal removed the cause of dilatation of the denervated pupil which occurs during muscular activity. On the other hand the denervated pupil could be made to dilate by painful stimulation (tetanizing current applied to saline soaked ear) or by partial asphyxia (occlusion of trachea).

In another cat which usually gave a good dilatation of the denervated pupil beginning five minutes after the work in the mill started and gradually increasing throughout the test, after the removal of one adrenal and denervation of the other, repeated tests failed to give the denervated pupil reaction. However, one hundred and sixty-eight days after the operation the fatigue test again elicited dilatation of the denervated pupil although the reaction developed more slowly and less intensively than before. The adrenal was again exposed and found to be connected with nerve fibers from the splanchnic nerve. These were destroyed. Later repeated fatigue tests failed to elicit dilatation of the denervated pupil.

Knowing the affect of epinephrin upon the denervated pupil it is most reasonable to suppose that the dilatation of the denervated pupil during work is due at least in large part to an increase in the epinephrin output.

Relation of the denervated pupil reaction to the work performed. A cat which shows a good dilatation of the denervated pupil accompanying work in the treadmill is able to do more work than it can when such dilatation is absent.

Although dilatation of the denervated pupil occurred with great difficulty in cat 101, bursts of fast work were usually accompanied by dilatation of the denervated pupil. Moreover the animal was able to travel only 728 m. when there was no accompanying dilatation of the denervated pupil, while on the same day when a certain amount of denervated pupil dilatation accompanied the test, the animal travelled 1438 m.

Cat 103 likewise was able to travel only 855 m. when no dilatation of the denervated pupil accompanied the test, but in the afternoon of the same day was able to go 1800 m. when there was some dilatation of the denervated pupil. In a similar manner spurts of work were attended by increases in the dilatation of the denervated pupil. This animal later (two hundred and fifty-seven days after cutting nerves to adrenal) recovered the denervated pupil response so that it was easily elicited in the treadmill. The farther it travelled the greater became the dilatation. At this time it was able to travel 3720 m. in the treadmill and there was no sign of convulsions, in fact it could have gone farther. This striking change can only be accounted for by a regeneration of the nerves to the adrenal.

Whenever Cat 105 worked well in the treadmill the denervated pupil became dilated, especially accompanying spurts of work. From the time that the animal was first tested as to the pupil reaction (fifty-three days after removal of one adrenal and cutting the nerves to the other) the denervated pupil always showed some response which we thought might be explained by regeneration of the nerve fibers. At autopsy (sixty-one days after adrenal operation) this appeared to be the case because nerve fibers again connected the semilunar ganglion with the adrenal.

Cat 111 also demonstrates the intimate association of work power with adrenal activity. At first when the adrenals were intact every test, except one, elicited a good pupil reaction. In this one test the pupil reaction was entirely absent. The cat was able to travel only

741 m. because of the appearance of convulsions. But on the afternoon of the same day in which this test was made, the cat when tried in the treadmill gave an excellent pupil dilatation and travelled 2240 m. Even that was not the limit of possible work. The denervated pupil dilated more and more as the work progressed until the stage of marked dilatation was reached. Spurts of work were attended by increases in the dilatation. Soon after removal of the right adrenal and of the left semilunar ganglion, the cat refused to work well at any time, likewise dilatation of the denervated pupil was absent. However, one hundred and sixty-eight days after the latter operation work in the treadmill was accompanied by moderate dilatation of the denervated pupil. The accompanying work was an improvement but not so good as that obtained before the adrenal operation.

The remaining adrenal was again exposed. Nerves had regenerated from the splanchnic to the adrenal. These were cut. Tested later, the animal refused to work well although sufficiently to elicit a good dilatation of the pupil under normal conditions, yet dilatation was entirely absent.

In another cat which possessed but one adrenal and that denervated, a good eye reaction was obtained accompanying work. At autopsy, nerve fibers were found connecting the splanchnic with the adrenal.

Cats possessing at least one normal adrenal give dilatation of the denervated pupil after working in the treadmill a short time. This was true for 13 normal animals which we tested. In only two cats, one instance in each case, did such dilatation fail to appear. However these cats when tested at other times gave good dilatation of the denervated pupil.

Denervated eye reaction in convulsions caused by fatigue. Most cats, which work hard in the treadmill and at the same time give a good denervated pupil dilatation, show no ill effects beyond a marked appearance of fatigue even if they travel far. On the other hand when the denervated pupil fails to dilate during the development of fatigue, if the animal is required to travel far, convulsions often occur. At least these are the results in our cases.

In cats with intact adrenals fatigue convulsions are difficult to obtain. One good example of such a case however is found in our cat 111 in which both adrenals were intact. On March 19, at 12 m. the cat travelled 780 m. in forty-one minutes giving a good dilatation of the denervated pupil. At 3 p.m. of the same day a ten minute test of 111 m. likewise produced a good denervated pupil dilatation.

Two days later at 9 a.m. the cat was required to travel 741 m. in fifty-one minutes, the test being terminated by convulsions. There was no dilatation of the denervated pupil throughout the test. Seven hours later, on being tested in the treadmill, the denervated pupil began to dilate after the first few minutes of work, becoming larger and larger as time went on. At fifty-six minutes there was a very active period in which the animal paced very rapidly for many revolutions. This was accompanied by a still greater dilatation of the denervated pupil. The animal continued to work until it had travelled 2240 m. in two hours, the denervated pupil being extremely dilated at that time. The cat was now weak but could have continued its work longer. This animal travelled faster and three times farther when there was a good dilatation of the pupil as compared to the test with absence of denervated pupil dilatation. Moreover convulsions appeared in the latter but were entirely absent in the former test. Repeated attempts have not been able to produce convulsions in this animal since the time mentioned above.

In some cats with but a single adrenal, and that denervated, convulsions were very easily produced by fatigue. (See protocols for cats 101 and 103.) In cat 101, five out of eighteen tests ended in convulsions and in only one of these, the last, was there any evidence of dilatation of the denervated pupil (i.e., so that the denervated pupil was larger than the control). This last test was made two hundred and seventy days after the primary adrenal operation, allowing ample time for regeneration of nerve fibers and perhaps accounting for the dilatation which did occur. This dilatation, however, was very tardy and not very marked.

In cat 103, out of six tests within sixty-four days following the adrenal operation, three were terminated by convulsions. In none of the three was there dilatation of the denervated pupil. Yet two hundred and five days later, this cat travelled 3720 m. in one test without showing any evidences of convulsions. This is nine times as far as it usually travelled in the tests during the period when convulsions were easily produced. This test was accompanied by a very marked dilatation of the denervated pupil. Ample time for the regeneration of nerve fibers had elapsed.

All cats with a single adrenal and that denervated do not give convulsions upon testing in the treadmill. Some lack aggressiveness to the extent that they cannot be made by any means to work hard. If the animal does not work hard the conditions which produce convul-

sions never develop. This is true even in the entire absence of adrenals. Here the animal which we have tried lacks the staying power to carry it to the point of fatigue where convulsions might develop. Our observations lead us to the conclusion that fatigue accompanied by well marked dilatation of the denervated pupil is scarcely ever followed by convulsions.

Fatigue convulsions. The convulsions which resulted from work in the treadmill were caused by fatigue and not by dizziness. As proof that the latter was not a factor, cat 103, at the period when convulsions were easily produced, was tied to the tread of the mill, the mill then being rotated. The cat with head free was enclosed by a bag so that it could obtain the visual effects of the movement. The cat was rotated 1250 m. in seventy-one minutes. At no time was there any sign of discomfort, and there was never an indication of convulsions. We have conclusive evidence as to the cause of these convulsions, in the fact that they are never obtained except following hard work.

Although fatigue appears to be the cause, we do not know the manner in which this brings about convulsions. Perhaps fatigue products play a part in their onset because even though you stop the work immediately upon the first sign of twitching, the convulsions develop to their maximal extent. (See protocol, cat 103.) Or it might be a more purely nervous phenenomon, with fatigue products as accessory factors.

The usual course of events is as follows: The cat begins to twitch and jerk in a few muscles; this activity spreads and the intensity increases until virtually the whole musculature undergoes spasmodic contraction, the animal lying on its side. There is also marked salivation, maximal dilatation of both pupils and dyspnea accompanying the later stage. The duration of the convulsions is usually about ninety seconds. Convulsions are followed by nervousness in some cats but with little apparent change in others except the prostration of fatigue. No ill effects seemed to result in any instance.

We have been able to produce these convulsions in but two normals and only once in each. Two other cats showed convulsive movements but not the typical convulsions with prostration. In all we have tested 19 normal animals. In the two instances of convulsions the denervated pupil was smaller than the control up to the appearance of convulsions. These two cats gave good dilatation of the denervated pupil in other tests and there were no convulsions.

Thirteen of the 19 normal animals had the superior cervical ganglion removed on one side. During work in the treadmill all of these gave

greater dilatation of the denervated pupil than the control except in the two instances above mentioned.

Of five animals which possessed but a single adrenal, and that denervated, two (no. 101 and no. 103) gave little or no dilatation of the denervated pupil when worked in the mill and in these convulsions could be obtained repeatedly by fatiguing in the mill. Convulsions were never caused in the other three, one always responded by a good denervated pupil reaction while the other two refused to work very hard. One of the latter also gave a fair denervated pupil reaction at times, while the second did not have a denervated pupil.

Because convulsions appeared only when there had been little evidence of epinephrin preceding them and because they appeared to be due to something suddenly released into the system, we have attempted to produce convulsions in animals by the injection of toxic doses of epinephrin.

Lethal doses of adrenalin were injected into two kittens and one adult cat but no evidence of convulsions appeared in any one of these.

Two guinea pigs were tried as follows:

Protocol, guinea pig, 695 grams

a.m.
10:25. Injected 0.1 cc. 1:1000 adrenalin into ear vein.
10:31. Injected 0.1 cc. 1:1000 adrenalin into ear vein.
10:34. Injected 0.15 cc. 1:1000 adrenalin into ear vein.
10:36. Spasmodic breathing, gasping but no convulsions.
10:39. Still breathing in gasps.
10:41. Died. No convulsions.

Protocol, guinea pig 700 grams

10:49. Injected 0.1 cc. 1:1000 adrenalin into ear vein.
10:50. Begins to quiver.
10:54. Does not move quickly when touched.
11:06. 0.1 cc. 1:1000 adrenalin injected into ear vein.
11:07. Lies down.
11:09. Dyspnea.
11:23. Restless.
11:25. Few spasmodic contractions of whole body.
11:28. Spasmodic contractions again.
11:30. Spasms more pronounced.
11:31. Urinated.
11:35. Spasmodic contractions, which recurred at intervals of 2 or 3 minutes until they become quite severe.
p.m.
12:08. Spasms less frequent and less severe.
12:33. Spasms still appearing.
Animal recovered.

On the next day, 3 intravenous injections (1:1000 adrenalin) of 0.1 cc. each given at 11:36, 11:41 and 11:43 a.m., respectively, failed to produce spasms. It seems impossible that epinephrin might be suddenly increased to this extent in animals having but a single, more or less denervated adrenal. Likewise epinephrin convulsions seem to be rarely obtained.

Protocol, cat 101. After having kept this animal under observation for one month, the right adrenal and the left semilunar ganglion were removed by the lumbar route. Before the operation, tests were carried out in the treadmill but not to the point of great fatigue, e.g.., in one test 370 m. were covered in 5.5 minutes, in another 411 m. in 10 minutes.

17 days after operation. Wound healed, cat travelled 332 m. in 6 minutes. Tired.

18 days after operation. Travelled 180 m. in 5.5 minutes.

19 days after operation. Travelled 358 m. in 12 minutes; so completely fatigued that it could neither keep pace nor hold on to the tread.

20 days after operation. Travelled 488 m. in 17 minutes.

21 days after operation. Travelled a total of 525 m. in two work periods. Approximately equal in time (5 minutes) and distance but 30 minutes rest between.

23 days after operation. Travelled 1590 m. in three periods of about 19 minutes each and one had 4 hours rest periods between.

25 days after operation. Travelled 515 m. in 10 minutes after 30 minutes rest, travelled an additional 515 m. in 16 minutes. The second period was terminated by *convulsions* following which the cat raced around the room four times and then relaxed, tremors persisting in the forelegs.

27 days after operation. Travelled 528 m. in 18 minutes.

30 days after operation. Travelled 764 m. in 16 minutes *convulsions* terminating the test.

32 days after operation. Travelled 755 m. in 18 minutes.

47 days after operation. Travelled 423 m. in 6 minutes. Two hours later travelled 545 m. in 15 minutes. Seven hours after first test, cat travelled 473 m. in 28 minutes, *convulsions* at close.

52 days after operation 1, removed the left superior cervical ganglion.

65 days after primary operation again tested in the mill. At 9 a.m. after 21 minutes, 660 m. the cat held weakly to the slats being unwilling to make the effort of pacing.

At 26 minutes, 728 m. very marked convulsions which lasted 90 seconds. After convulsions had ceased the cat whined and was extremely irritable so that if disturbed she jumped about irrationally. When undisturbed she showed spasmodic jerks. There was no dilatation of the denervated pupil up to the time of the convulsions.

At 3:30 p.m. cat again in treadmill. This time the denervated pupil occasionally dilated to the size of the control pupil. The animal after 16 minutes, 302 m., could do nothing but slide and hold feebly to the slats until 1 hour, 1300 m., when there was a burst of working power, the cat pacing 138 m. very rapidly without cessation. Following this the denervated pupil was larger than the control. Test stopped at 70 minutes, the animal refusing to work further.

Seventy-six days after primary adrenal operation, adrenal and lumbar veins to the remaining adrenal, tied.

This cat became pregnant, six healthy kittens being born one hundred and eighty-two days after the primary operation.

Two hundred and seventy days after primary adrenal operation. Tested in the treadmill. After 5 minutes, 155 m., denervated pupil still smaller than the control. After 10 minutes, 333 m., denervated pupil just larger than the control. At 843 m. there was a spurt of fast pacing continuing for 104 m. when the animal was seized with convulsions. Pupil reaction was very slow to develop. The animal did not appear to be as aggressive as it had formerly. It really worked well only at the last spurt.

Protocol, cat 103. Removed right adrenal, cut nerves to left adrenal. Forty-three days later, destroyed left superior cervical ganglion. Fifty-seven days after adrenal operation, tested in treadmill. Paced practically all of the way, going 476 m. in 14 minutes. There was no dilatation of the denervated pupil throughout until the convulsions which terminated the test. Convulsions were accompanied by urination and defecation. Animal very irritable afterward.

Fifty-eight days after primary operation. *Tested at 8:25 a.m.* Worked hard in the mill; tried to avoid pacing but did slide. No dilatation of denervated pupil. Convulsions at 855 m., 27 minutes.

Tested at 4 p.m. Weak, hangs to tread and drops or jumps much of the time. No dilatation of denervated pupil until 80 minutes, 1661 m. when there was a spurt of fast pacing (111 m. in 2 minutes). This was accompanied by enlargement of the denervated pupil to an equality with the control. Spurts of work occurred from time to time after this, being accompanied by a similar dilatation of the denervated pupil. Stopped at 96 minutes, 1800 m., the cat raced wildly about the room and then lay down. Tested the next morning the cat refused to work hard at any time. There was no dilatation of the denervated pupil.

Sixty-four days after primary operation. Tested in treadmill for 33 minutes, going 1020 m. No dilatation of denervated pupil throughout the experiment, which was terminated by convulsions lasting 85 seconds. Very nervous afterwards. The treadmill was being turned very slowly and was instantly stopped at the first sign of twitching. In spite of this, the twitches and jerks became gradually more severe until violent convulsions had developed. It seemed that once the threshold was crossed convulsions developed.

Two hundred fifty-seven days after primary operations. Tested in treadmill, denervated pupil larger than control at 6 minutes, 136 m. At 17 minutes, 370 m., denervated pupil very much larger than control. At 27 minutes pacing unusually fast and well ("second wind"). At 35 minutes, 1000 m., cat still travelling.

Two hundred sixty-nine days. Tested in treadmill.

After 3 minutes, 87 m., denervated pupil just larger than control.

At 20 minutes, 690 m., denervated pupil just larger than control.

At 35 minutes, 1120 m., denervated pupil decidedly larger than control.

At 83 minutes, 2600 m., denervated pupil much larger.

At 120 minutes, 3500 m., denervated pupil very much larger.

At 131 minutes, 3720 m., denervated pupil very much larger than control.

Although the animal was tired it could have travelled farther.

Discussion. In experiments of long duration, dietary factors must be carefully ruled out by feeding a variety of foods to assure a plentiful supply of the different vitamines. For example, we found that boiled liver and whole cow's milk lacked something for the proper growth of kittens, although adult cats apparently thrived on this diet. The addition of yeast made the diet adequate for kittens and was therefore used in the adults.

Occasionally mange gets a start among the cats and is readily spread. This can be checked by daily soaking of the hair and skin with a saturated solution of chloramine-T (4) on the ears, head and neck. Mange in the cat is first detected by the presence of tiny lumps in the skin on top of the head or neck or sometimes on top of the ears.

We also found it advisable to remove fleas from cats newly arrived at the laboratory by giving them a good soaking in a dilute solution of "Coronoleum" (West Disinfecting Company). By thoroughly drying the animal immediately afterward most of the "Coronoleum" is evaporated and the animals are less likely to be poisoned by licking the fur.

Our results indicate that the dilatation of a denervated pupil can be used as a test of increased adrenal secretion in muscular work, for removal of both adrenals or cutting the nervous connection with a single remaining adrenal abolishes the dilatation which accompanies muscular work. On the other hand such dilatation again reappears after regeneration of the nerve fibers.

Removal of one adrenal and cutting the nerves to the other as shown first by Elliott (5) and later by Stewart and Rogoff (2) certainly has shown that central nervous control of the adrenal is not necessary to maintain the vital processes. But our observations seem to indicate that this nervous control bears an important relation to the muscular efficiency of the animal. Tests of this must be made before the nerve fibers have time to regenerate. For if the nerves are cut in the abdominal cavity of the cat the central end has but a short distance to grow to reach the adrenal. It has been shown by Langley (6) that nerve fibers in the cervical sympathetic regenerate at a fairly rapid rate. If this rate holds for the splanchnic it should not take many days for some of the fibers to reach the adrenal, the distance is so short. That such regeneration does take place is indicated by our observations on the return of the denervated eye reaction to fatigue. Examination of our animals, in which we have suspected nerve regeneration, has shown that nerve fibers have again connected with the adrenal. Cut-

ting these fibers has again removed the nervous control of the adrenal as indicated by the denervated eye reaction in fatigue.

Of course many fibers no doubt fail to find their way back or do so after a very long interval so that the nervous control would be completed only after a prolonged period. However those that do begin to function are factors which must be considered in adrenal activity.

We are yet in the dark as to the nature of fatigue convulsions in cats. Because of the fact that these can occur in normal cats and that they do not occur in cats with a single denervated adrenal until some time after cutting the nerves (such a time as might permit regeneration of some nerve fibers), the rather remote possibility that they might be due to a sudden release of epinephrin suggested itself. In reviewing the literature covering the toxic effects of epinephrin we have found that epinephrin convulsions have been described by Batelli (7) and Schaller (8). The former says that they are frequent in animals before death from epinephrin. The latter said that he frequently produced typical epileptic attacks in rabbits from maximal sub-lethal injections of adrenalin.

On the other hand Foà and Pellacani (9), Oliver and Schäfer (10) and Vincent (11) described dyspnea, circulatory effects and prostration as the typical results following toxic doses of adrenal extracts. We have also found convulsions rarely produced by epinephrin injections.

Furthermore it is difficult to conceive of an arrangement which would permit a sudden flooding of the circulation with epinephrin. It is true up to the time of fatigue convulsions in our experiments, epinephrin is not poured into the circulation in sufficient amounts to affect the denervated pupil to any extent. On the other hand whenever the denervated pupil gives an indication of a steady outpouring of an increased supply of epinephrin, fatigue convulsions usually have not occurred.

Fatigue convulsions never develop unless the animal works hard, at least for a short time. Thus we have been unable to produce them by leading a susceptible cat (no. 103) around the room for 3400 m. (the cat did become tired but not greatly fatigued). The work performed in this test is a great deal less than that performed over a much smaller distance in the treadmill for the cat was supported to a considerable extent by a harness around the shoulders, otherwise it refused to travel. Likewise we were not able to produce convulsions in a cat from which both adrenals had been removed. This cat, however, did not work hard.

Observations from many sources point to a close relationship between the adrenals and muscular activity. First, the muscular weakness of adrenal insufficiency suggests an intimacy between the two tissues; second, the vacuolization of the adrenals after prolonged muscular activity (12); third, the size of the adrenals increases with the development of the muscles (13), (14).

A great deal of work has been done which shows that epinephrin may be of importance in muscular fatigue. The quantity of epinephrin in the suprarenals is very much decreased by great muscular fatigue (15). This has a great deal of significance when connected with the observation that epinephrin delays the onset of fatigue in skeletal muscle (16), (17), (18).

The beneficial effects of epinephrin in fatigue may be due not only to an increase in the circulation (19) to the muscle but to some other action yet undetermined (18).

Our observations furnish evidence of the increase of epinephrin during fatigue in normal unanesthetized animals. They show further that the absence of such an increase reduces muscular efficiency.

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SUMMARY

1. Normal cats usually give a dilatation of the denervated pupil accompanying work in a treadmill. This begins within a few minutes and increases as the work progresses. Spurts of work are accompanied by greater increases in the dilatation; the same cat will work harder and travel farther when such dilatation is present. If dilatation is absent and the animal works hard convulsions may follow but they usually do not result if there is a good dilatation of the denervated pupil.

2. The dilatation of the denervated pupil accompanying fatigue is absent in animals deprived of both adrenals or possessing but a single adrenal and that completely denervated. Such dilatation, therefore, is probably caused by epinephrin.

3. Cats possessing but a single adrenal, and that denervated, undergo a period of ill health which appears to be more marked than that of control animals. This is indicated by the loss of appetite, loss of weight, change of temperament, weakness and in some instances a subnormal temperature. During this period the working power is very much decreased. On the other hand, after regeneration of some of the nerve fibers, health and power to work are regained.

4. Certain cats possessing but a single adrenal, and that denervated, developed convulsions in the treadmill repeatedly. Preceding these, dilatation of the denervated pupil was scanty or absent. In the cat which developed these convulsions most easily, they could be obtained with difficulty after regeneration of a large number of the nerve fibers to the adrenal judging from the denervated pupil reaction. At this time the animal could travel many times farther in the treadmill than before regeneration of the nerves.

5. Our results seem to indicate that epinephrin plays a very important rôle in increasing muscular work and delaying the onset of fatigue.

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SENSORY STIMULATION BY UNSATURATED ALCOHOLS, POLYHYDRIC ALCOHOLS AND CHLORHYDRINS

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It is commonly stated that in many respects the unsaturated monohydric alcohols resemble the saturated; and that in each series of polyhydric alcohols the members act much alike, and that to a considerable extent their chlorhydrins behave like them. It is of interest to see how much correlation exists between the chemical behavior of such organic substances and their action on living protoplasm.

The efficiency of such alcohols as allyl alcohol, ethylene glycol, glycerol and of the chlorhydrins, ethylene chlorhydrin and glycerol chlorhydrin, was tested by noting their effect on the sensory cells of *Allolobophora foetida*. These results are to be compared with the effects of monohydric alcohols which were studied in a previous investigation (1).

The method used was the same as that described in a previous paper (2). When a worm is placed on a table, surrounded by a test solution, the worm will slowly crawl to the edge of the table and enter the solution. The time elapsing from the moment the prostomium enters the solution until it is withdrawn is the reaction time of the worm to the test solution.

Comparison of figure 1 with figure 2 shows that the efficiency of allyl alcohol is much greater than that of either glycol or glycerol. The behavior of allyl alcohol is more comparable with that of propyl alcohol than with that of the polyhydric alcohols. Glycol, which has one carbon atom and one OH group less than glycerol, is more efficient than glycerol, though both glycol and glycerol are very inefficient as compared to allyl alcohol.

Since high concentrations are used, the effect of glycol and glycerol might be interpreted as due entirely to the extraction of water. However, on comparing the osmotic pressures of glycol and glycerol it seems that the effect cannot be entirely due to the extraction of water, since at the concentration of these two alcohols which bring about the same reaction time, the osmotic pressures of the alcohols are different.

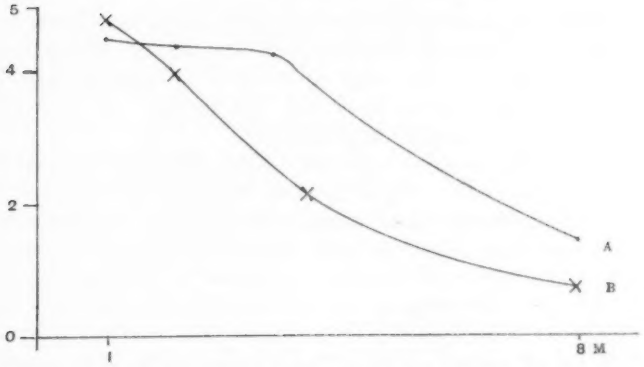

Fig. 1. The effects of ethylene glycol (curve *B*) and glycerol (curve *A*) as stimuli for worms. The reaction times in seconds are plotted as the ordinates, and the molar concentrations as abscissae.

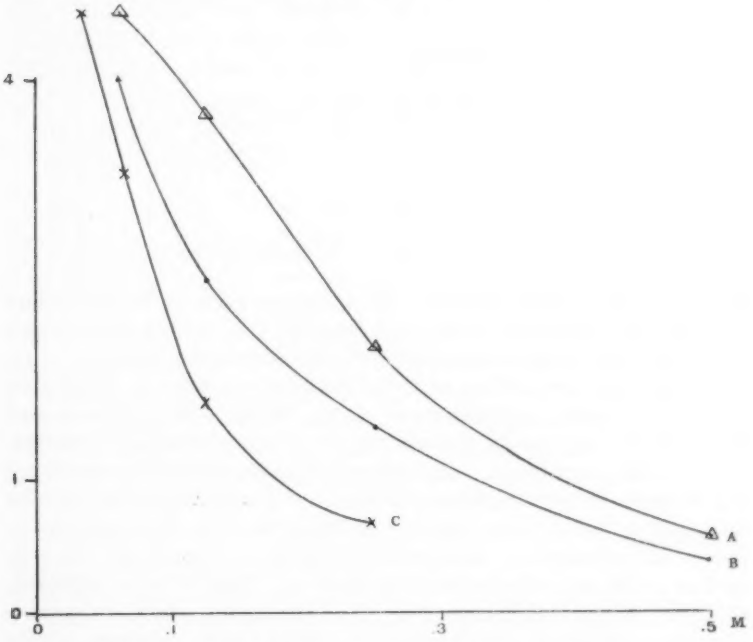

Fig. 2. Efficiency of allyl alcohol (curve *A*), ethylene chlorhydrin (curve *C*), and glycerol monochlorhydrin (curve *B*), as stimuli for worms. The reaction times in seconds are plotted as the ordinates, and the molar concentrations as abscissae.

It is commonly stated that in many respects the increase in the number of hydroxyls is of no great consequence in the ordinary chemical reactions of such alcohols, but the case is different as regards their effects on the sensory cells, since the more OH groups, the less efficient the alcohols are in producing stimulation.

As for the chlorhydrins, ethylene chlorhydrin and glycerol monochlorhydrin were used. Figure 2 shows that glycerol monochlorhydrin is slightly less efficient than ethylene chlorhydrin. Greater difference is found, however, when glycerol monochlorhydrin is compared to glycerol, and ethylene chlorhydrin is compared to ethylene glycol. Replacement of one OH group by one chlorine atom increases the efficiency several times. The H-ion concentrations of the two chlorhydrins were carefully tested; it was found that this increase in efficiency is independent of the H-ion concentration. This increase may be due to the fact that the chlorhydrins hydrolyze on entering the sensory cells. Such a hydrolysis has been described by workers on mustard gas (3).

TABLE 1

Narcotic action

SOLUTION	CONCENTRATION BELOW WHICH NO CESSATION OF MUSCULAR ACTIVITY OCCURS IN 1 HOUR AT 23°C.
Allyl alcohol	0.5 M
Ethylene chlorhydrin	0.5 M
Glycerol monochlorhydrin	0.5 M
Ethylene glycol	2 M
Glycerol	1 M

As for the narcotic effect of the given reagents, table 1 shows that allyl alcohol and chlorhydrins are more efficient than glycerol and glycol, which agrees with the stimulatory effects of the same reagents, as previously mentioned. One other distinction should be mentioned here between these two groups of reagents. The limiting concentration necessary to bring about narcosis is higher than the limiting concentration for stimulation. This is true for saturated monohydric alcohols as well as for allyl alcohol and chlorhydrins. This is to be expected, since for sensory stimulation only the prostomium, which is the most sensitive portion of the worm, is involved, while narcosis affects the entire body of the worm, which decreases in sensitivity posteriorly.

On the other hand, with polyhydric alcohols (glycol and glycerol) the limiting concentrations are not higher for narcosis than for stimulation; in the case of glycol they are the same as for stimulation and in case of glycerol they are lower than for stimulation. This distinction may be due to the fact that the effect of the former group depends primarily on chemical action while the effect of the latter group depends partly on chemical action and partly on the extraction of water. This supposition is rendered probable by the following facts. At the concentration in which the reaction time is about two seconds, there is no narcosis when saturated monohydric alcohols, allyl alcohol and chlorhydrins are used, while there is narcosis when glycol and glycerol are used. When the worm thus narcotized by glycol or glycerol is removed from the solution and placed in water, the motility of the worm is resumed very slightly for a time but this is followed in many cases by death. This inhibition of motion is perhaps due to the extraction of water from the body; although the motility of the worm is recovered to a very slight extent after the worm is replaced in water, the extraction of water has apparently gone so far that recovery is not possible. Thus it seems that the apparent narcosis due to the effects of glycol and glycerol on the worm may depend to a great extent on the extraction of water and not on chemical action.

SUMMARY

The experiments show that while the behavior of allyl alcohol toward the sensory cells of the worm is just what would be expected on the ground of its ordinary chemical relations, this is not the case with polyhydric alcohols and chlorhydrins. From a chemical standpoint, we should expect no such difference in behavior among the polyhydric alcohols themselves (and this would also apply to their chlorhydrins) as we find that in sensory stimulation where the efficiency decreases with the addition of OH groups, and a great increase in efficiency occurs when an OH group is replaced by a chlorine atom.

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SUCCESSIVE STIMULATION BY ALCOHOLS

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In a previous paper (1) it was shown that in successive exposures of *Allolobophora fœtida* to a salt solution (such as potassium chloride) an increase in sensitivity took place at certain concentrations. This was interpreted to mean that there was a definite combination of the stimulating reagent with some constituent of the sensory cell.

It is of interest to investigate the action of alcohols from this point of view. For this purpose the effects of a series of monohydric saturated alcohols, methyl alcohol, ethyl alcohol, n-butyl alcohol and isoamyl alcohol have been studied.

Successive contacts of the prostomium of the worm with a solution of alcohol may bring about a definite change in sensitivity which depends upon the concentration. With the alcohols studied it was found that if we employ a concentration giving a reaction time of less than one second, a decrease of sensitivity takes place as the result of successive exposures. This is evident from the gradual lengthening of the reaction time, as shown in figure 1.

At a concentration lower than this, a shortening of the reaction time (increased sensitivity) is observed after a few successive exposures, as shown in figure 2. At a still lower concentration (as shown in fig. 3) there is no change in the reaction time, though the prostomium of the worm is repeatedly exposed.

We may account for these facts by supposing that the alcohol, A, unites with a substance, X, in the prostomium to form a compound AX, which is necessary for stimulation. On this basis we should expect the reaction time to shorten as the concentration of the reagent in the solution increases.

It is evident that no stimulation occurs until this compound reaches a definite concentration (threshold effect). It is also evident that this compound tends to disappear, since otherwise the weakest concentrations of alcohol would stimulate in the course of time, which is not the case.

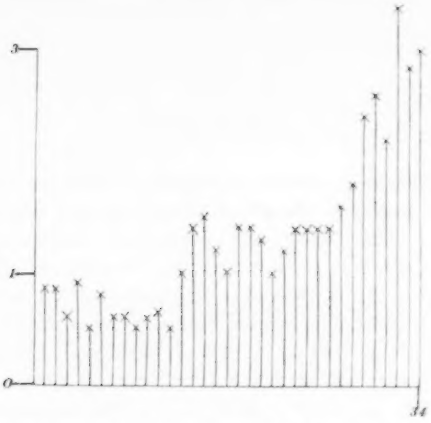

Fig. 1. Successive reactions of worms exposed to 1 M ethyl alcohol. Reaction time in seconds is plotted as ordinates, successive exposures as abscissae. This shows the lengthening of the reaction time which is characteristic of high concentrations of alcohol.

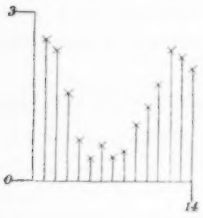

Fig. 2. Successive reactions of worms, showing an initial decrease in reaction time followed by an increase. This effect is characteristic of concentrations of alcohols somewhat lower than those which give the effect shown in figure 1. The data were obtained with 0.5 M ethyl alcohol. The reaction times in seconds are plotted as ordinates and successive exposures as abscissae.

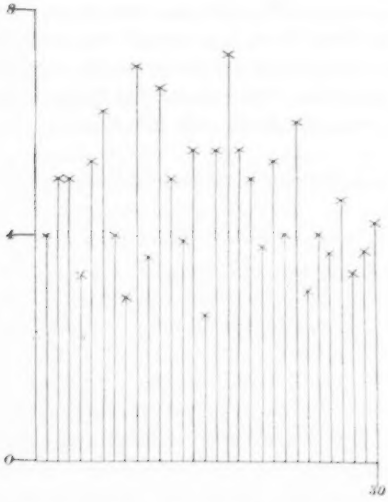

Fig. 3. Successive reactions of worms showing no change in the average reaction time. This effect is characteristic of concentrations of alcohols lower than those which give the effect shown in figure 2. The data were obtained with 0.25 M ethyl alcohol. The reaction times in seconds are plotted as ordinates and successive exposures as abscissae.

After any exposure a certain amount of alcohol remains in the tissue and this must augment the effect of the alcohol which enters at the next exposure. Hence the reaction time will shorten, unless the concentration of alcohol is so low that the compound AX disappears as fast as it is formed, so that the augmentation is ineffective. As a matter of fact we find that the reaction time does shorten in case of the above experiments as well as in case of those with the potassium salts.

The rate of formation of AX depends on the concentration of both A and X and it is therefore evident that as the substance X is used up the reaction time must lengthen.

If the concentration of alcohol is high enough X is used up so rapidly that the reaction time begins to lengthen after a few exposures have been made.

If there is a sufficiently long interval between exposures, X is more or less completely restored and a corresponding recovery of sensitivity will take place.

This hypothesis seems to afford a simple explanation of the facts without involving any unreasonable assumptions.

SUMMARY

1. A series of monohydric saturated alcohols was used for a repeated stimulation of worms at different concentrations. In the case of each alcohol it is found that there is a certain concentration at which successive exposures bring about a decrease in the sensitivity of the worm, concentrations somewhat lower than this bring about an increase in sensitivity, and concentrations still lower bring about no change in sensitivity.

A hypothesis is developed to account for these facts.

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THE EFFECT OF VARIOUS SALTS ON THE OUTGROWTH FROM EXPERIMENTAL AMOEBOCYTE TISSUE NEAR THE ISOELECTRIC POINT AND WITH THE ADDITION OF ACID OR ALKALI

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The starting point of our investigations was observations which we made in the year 1905 and 1906 (1). At that time we observed that in isotonic NaCl solutions Limulus cells sent out pseudopodia, but soon lost their granules and became hyaline. In acid and alkali solutions the cells rapidly took up water, swelled and lost their granules; in addition there were in acids some changes which were due to coagulative processes which were lacking in alkali. Thus neutral isotonic NaCl solutions as well as acids and alkali were more or less injurious. If, however, acid or alkali and neutral isotonic NaCl solutions were combined, they mutually counteracted their injurious influence and the cells remained well preserved. Both, small amounts of acid and alkali had this effect. HCl was in this respect much more potent than acetic or citric acid in accordance with the greater electrolytic dissociation of HCl. The fact that both acid and alkali were beneficial, while at the neutral point the cell granules were dissolved, we referred tentatively to the amphoteric character of the cell proteids (1907). Subsequently Michaelis and Takahashi (2) found that hemolysis of erythrocytes under the influence of acid takes place at a pH which represents the optimum for the coagulation of the stroma particles. This corresponded to the isoelectric point of the stroma. Jodlbauer and Haffner (3) studied hemolysis by heat, narcotics and hypotonic substances and interpreted their results in a similar way. R. Hoeber and R. R. Spaeth (4) likewise referred quantitative relations which they observed in analysing the effect of certain trivalent kations on the contractility of muscle to the variation in the electric charge of the colloids, and to a greater liability to injury of the solutions at the isoelectric point.

As to the antagonism between neutral salts and acids and their mutual neutralizing effects, a considerable number of important results has been recorded. Jacques Loeb (5) first observed in 1899 that acid increased the affinity of muscle for water. Neutral solutions of NaCl also favored water absorption by muscle; but if acid was added to a hypertonic solution of NaCl, it caused a diminution in the taking up of water. Alkalies on the other hand always increased the absorption of water. The antagonism between acid and salts was in later years studied by R. S. Lillie (6) in its effect on the ciliated epithelium of Mytilus, by Jacques Loeb under varied conditions in its effect on embryonic and adult Funduli (7). W. J. V. Osterhout (8) determined variations in the electrical resistance of Laminaria under the influence of different electrolytes and with this method showed that also in the case of plants an antagonism exists between acids and neutral salts which affects primarily the permeability of the cells.

Of special interest is the observation of this author that acids decrease and alkali increases the permeability of Laminaria. These authors agree that the antagonism between neutral salts and acids depends upon the effect of these substances on the cell membranes.

The most recent studies are those of M. E. Collett (9), who found similar antagonisms in the effect of salts and acids in the case of Infusoria. Some of her conclusions very closely parallel our earlier observations on the amoebocytes of Limulus.

Our present investigations carry further our earlier work. We studied in vitro the effect of solutions of various neutral salts on the cultures of experimental amoebocyte tissue with and without the addition of variable amounts of acid and alkali. After preliminary experiments had demonstrated (10) that cells may show very active migration in such acid solutions, sometimes surpassing those in neutral solutions, we now found that through both addition of acid and alkali to neutral solutions not only an extremely marked improvement in the outgrowth of the amoebocytes may be obtained, but that the outgrowth in acid may even surpass in extent and duration the outgrowth in Limulus serum which represents the natural and otherwise most favorable medium for the migration of the amoebocytes, and furthermore that solutions of neutral salts, which are an unfavorable medium for the amoebocyte tissue, may be converted into a favorable one by the addition of acid and, although less strikingly, by the addition of alkali.

1. *Method and variable factors.* The method used in these investigations is the same as in the two previous investigations. We prepared

experimental cell fibrin (Amoebocyte) tissue of Limulus and we placed small pieces of this tissue on cover glasses. We surrounded the pieces with fluid and inverted the cover glass over a hollow glass slide, fixing it to the latter with vaseline. We made thus tissue culture preparations of experimental Amoebocyte tissue.

In our preceding publications we called attention to some variable factors which may occur in such experiments. They are as follows: *a*, The size of the piece is of importance. We used on the average pieces about 3 mm. square; if the pieces are much smaller, the outgrowth is usually less extensive. The cut surfaces ought to be straight; irregular and torn outlines at certain places may signify that the tissue has been injured and diminished outgrowth may result from this injury. *b*, It is advisable to surround the pieces with a considerable amount of fluid. We used especially deep and wide hollow slides. If the amount of fluid surrounding the pieces is small, the effect of the various solutions may become obscured. Either firm, tissue-like or soft mucoid material can be used. Cell fibrin as it is obtained about 5 to 6 hours after withdrawal of the blood is usually soft, and it has to be scraped off the dish in order to place it on the cover glass. On the following day such material is usually tissue-like and can be cut into regular pieces; but sometimes it is still mucoid even after having been kept 20 hours in the ice chest. Tissue 2 days old or older usually retracts when cut and generally is inferior to fresh tissue. In our recent experiments we found indications which pointed to the presence of an additional variable factor. In the two preceding summers (1919 and 1920) in the large majority of experiments the tissue grew well in isotonic NaCl solutions; on the second day the outgrowth in this solution not rarely surpassed that in Limulus serum, while the latter proved usually superior on the first day. In the experiments of the summer 1921 the tissue grew well in an isotonic solution of NaCl only in about 10 per cent of the experiments; in the large majority of the experiments the outgrowth in this solution was very slight or missing altogether; instead we found signs of the destruction and solution of the cells and of the margin of the piece in these salt solutions, although the experiments were carried out under sterile precautions. The difference is due either to a variability in the amount or character of substances extracted from the piece, or to variations in the resistance of the cells in different individuals. We have called attention to the possible influence of variations in the amount of serum or other substances extracted from the tissues in our previous paper. It seems most probable that the animals were less resistant in

1921. We obtained the best results in isotonic NaCl solutions in experiments in which the tissue was till loose and very sticky after having been kept in the ice chest for 20 hours or more. In such cases the cells evidently had less tendency to undergo those changes which lead to a retraction of the tissue. On the other hand, tissue which was equally loose only 5 hours after the withdrawal of the blood usually did not give better results, although it contained presumably a relatively great amount of serum. It is probable that under favorable conditions healthy cells migrating out of the tissue, aided perhaps by the presence of substances extracted from the tissue, can resist otherwise adverse environmental conditions. Thus we found in our previous experiments that even in hypertonic as well as in hypotonic NaCl solutions cells may migrate out of the pieces and that even in these solutions the character of the pseudopods may remain unaffected by the environment for some time. Only at certain periods in their history the cells show the typical changes. It is probable that the power of resistance varies in different animals.[1]

The effect of Limulus serum is much more constant. We must therefore in the first place compare experiments made with the same tissue, and only secondarily can we compare with each other the results obtained with different tissues.

The effect of Limulus serum. As in our previous experiments, Limulus serum proved to be the best medium in the first 24 hours. Later extension and gradual hyalinization of the cells occurred and this made an end to the further outgrowth. Limulus serum gave the most constant results; they were less affected by variations in the character of the tissue. Some variations however occurred even here; tissue which was still mucoid on the second day gave not only with isotonic NaCl solution but even with Limulus serum especially favorable results. This indicates that the beneficial results obtained with this tissue were in all probability not due to admixed serum. In Limulus serum the typical cell clumps formed and all varieties of tongue pseudopodia could be observed.

Even Limulus serum that had been kept by us for a period of about 11 months in test tubes, sealed with paraffin, and that had previously

[1] The winter and spring of 1920 and 1921 were unusually mild and it is possible that this climatic condition is responsible for the difference noted. It remains, however, for future investigations to determine which of the factors mentioned is responsible for this variability. It will also be necessary to consider the possibility that in some cases the blood was infected in the living animal.

been heated to 54° for half an hour, was found to be just as effective as fresh serum. As we stated previously, sera of other arthropods cannot take the place of Limulus serum. The effect of Limulus serum is therefore specific.

The effect of isotonic solutions (m/2 and 5/8 m NaCl). As stated above, in the large majority of the experiments, no or very little outgrowth was obtained in these solutions. Those cells that did grow out soon spread out and became hyaline and cytolysed. As a result of solution processes in the migrating cells or in the margin of the piece a finely granular precipitate often formed around the piece, or sometimes the viscous cell material was drawn out into radiating fibers which surrounded the margin of the piece. In other cases a layer of granular cells which were suspended in the fluid surrounded the piece. In several cases a slight outgrowth of granular contracted cells occurred. Gradually these cells spread out and became hyaline. In five experiments, however, a very good outgrowth was obtained similar to that recorded in previous years. In some of these cases it exceeded even the growth in Limulus serum especially on the second day.

These cells showed the characteristics formerly described. There was less agglutination in a m/2 NaCl solution than in Limulus serum. In the former the cells were on the whole more evenly distributed, although clumping did occur. The cells were more contracted and had somewhat sharper pseudopodia than in Limulus serum. Many cells showed in their posterior half furrows due to a localized contraction of the protoplasm. The cells being less sticky, a number of them detached themselves from the surface of the cover glass and fell down and formed a second layer of cells on the lower surface of the drop. This layer was usually smaller than the layer at the surface of the glass.

In the majority of our recent experiments the effect of an isotonic NaCl solution on the amoebocytes is in accordance with the result of those of our former experiments in which we received a drop of blood directly into a NaCl solution, or in which we poured an isotonic NaCl solution on the amoebocytes. Under those conditions we found the cells to send out rather sharp pseudopods and very soon afterwards to lose their granules and to become hyaline and cytolysed.

In order to explain the outgrowth of the cells in isotonic NaCl solutions, we had to assume that many cells growing out from the experimental amoebocyte tissue were protected in some way against the injurious effect of the NaCl solution, at least for some time. Later, however, they also degenerated. We considered previously the possi-

bility that substances might be extracted from the piece and that these protected the cells.

The effect of the addition of acid and alkali to the isotonic NaCl solution. The addition of acid to the isotonic (5/8 m to m/2) solution of NaCl caused in almost all experiments a marked improvement. One part of HCl solution varying in strength between m/10 and m/1000 was added to ten parts of the NaCl solution. The strongest concentrations caused a solution of the margin of the piece. The optimal action was obtained with m/100 to m/200 HCl. In the majority of cases m/100 HCl represented the optimal quantity; but in some experiments m/80 HCl likewise produced an improvement. In some cases m/200 lactic acid, m/200 HCl, or m/200 to m/400 butyric acid were optimal.

In the case of the organic as well as of the inorganic acids the optimum varied slightly in different cases, and in one case the optimal effect was found after addition of m/300 to m/400 acid (in the proportion of 1 part acid to ten parts salt solution).

Weaker solutions up to the strength of m/400 HCl still were advantageous; but usually the effect of weaker solutions was less marked than that caused by the optimal solutions. Still weaker solutions usually were without effect. In the fluid surrounding the piece the optimal concentration of acid corresponded therefore to somewhat less than m/1000 to m/2000 HCl solution; but a further deduction has to be made on account of a slight admixture of serum which was invariably adherent to the piece. It caused a precipitate with the acid and this precipitate surrounded the piece usually leaving a clear area directly around it. The proteid as well as inorganic buffer solutions contained in the admixed serum and dissolved parts of the tissue reduced, of course, the amount of free acid present in the fluid.

Not only HCl had this beneficial effect, but also organic acids, of which butyric and lactic acid were used. Although the electrolytic dissociation of the organic acids used is considerably below that of HCl, still their effect was very similar to that of HCl and they could be used in similar concentrations. Again addition of 10 per cent of a m/100 to m/200 solution of butyric acid and lactic acid was found best. We must therefore assume that acid does not altogether act through the changes in hydrogen ion concentration which it produces; but that the anion or the undissociated acid may play a certain part.

In a few cases no effect of the acid was obtained. These were cases in which only one concentration of the acid was tested and in which the observation extended only over the first 24 hours. In a complete experi-

ment a series of dilutions ought to be used and the observation ought to extend over several days. If that is done, positive results ought to be obtained in every case, except when the cells are already much deteriorated in the beginning of the experiment.

The results of addition of acid are most striking, when tissue is used which shows only a trace of growth or no growth at all in a neutral NaCl solution; then we find in many cases an excellent outgrowth in the acid solution which is often better than that in Limulus serum, especially on the second or third day; but even in those cases in which already in the neutral NaCl solution the outgrowth is very good, the acid produces improvement. This may be noticeable as early as 24 hours after the beginning of the experiment, but it becomes more marked after 2 or 3 days or even later.

At that time very decided retrogressive changes have taken place in the neutral solution, while in the acid the cells may still be very well preserved and active; new cells continue to move from the tissue while the cells which had already emigrated from the tissue extend still further into the surrounding fluid.

Through varying the strength of the acid we can produce graded effects on the tissue. If the amount of the acid added is very great (10 per cent of m/10 to m/40 HCl) solution processes take place in the tissue and in the cells; a few typical acid cells may be found. If the acid added is somewhat weaker (m/80 to m/100 HCl), the cells may at first appear as fixed oval or round granular cells with sharp margins. There may be a few sharp threadlike pseudopods or pseudopods may be absent altogether. Sometimes the effect of this solution is so strong that the cells remain in this fixed condition, but in other cases a slight movement of the amoebocytes out of the piece continues slowly, but steadily, and it may proceed for a considerable number of days We could follow it for 6 days; but there is very little doubt that under favorable conditions it may continue longer. The cells under those conditions are relatively contracted, show on the whole sharp pseudopodia and furrows in their posterior part. The granuloplasm is tight and moves connectedly and slowly into the pseudopods. If the optimal concentration is reached the growth is very extensive and active already on the first day. The character of the cells is on the whole the same; the cells are contracted, granular; their extension and hyalinization is very slight. On the first day addition of 10 per cent of a m/200 HCl may sometimes be as good as of a m/100 HCl solution; but on the second day the weaker solution usually allows a greater extension and subsequent hyalinization of the

cells. The weaker the acid is, the more it allows in the course of time extension and the degenerative processes which follow it to take place, although at first the acid may still exert a beneficial effect. A stronger solution usually shows more inhibition on the first day but it continues to keep the cells in a contracted and actively moving state for a longer period of time. It prevents very much more efficiently the spreading out of the cells and its injurious consequences.

In weaker acids the result is therefore more and more like that in neutral NaCl solutions; at first this weakening of the effect appears only after the lapse of some time; but if still weaker acid is used, it becomes apparent at once. The stronger solutions keep the cells contracted and, if used in optimal quantities, still sufficiently soft to permit active movement; if the acid action is still greater the cells become so hardened and fixed that, while extension is prevented, at the same time movement is also completely inhibited.

In accordance with this effect of acid on the consistency of the cells or its outer layer the pseudopods are generally made sharper through the addition of acid; occasionally, however, some drops are observed even in acid solutions, especially in the most advanced cells, which are exposed to the full effect of the solution.

Organic acids act on the whole like HCl; but in some cases it appeared as if possibly the cells were somewhat softer in the solutions of organic acids and some cells showed perhaps some broader pseudopods than in solution of HCl.

While thus acid in suitable concentration tends to preserve the blood cells, to prevent the spreading out of the amoebocytes and the retrogressive changes which follow this extension, this effect is after all not of indefinite duration, but after some time the cells will extend and hyalinize and become destroyed even in an acid solution.

The rapidity with which these secondary events occur depends upon the strength of the acid. If it is so strong that the migration is slow and the cells relatively little motile, as if it were hardened, the extension and subsequent retrogressive changes take place less rapidly; in weaker acid, as after addition of m/200 HCl, the extension and hyalinization usually occur more rapidly and may be noticeable already on the second day. Some cells, of course, in direct contact with the cover glass and situated in the periphery of cell clumps may extend and hyalinize as early as on the first day. But even extended cells may preserve their granules longer in acid than in a neutral solution.

The rapidity and intensity of outgrowth in an acid solution may be so great that it may surpass already on the first day the zone of growth in Limulus serum. It usually exceeds the growth in Limulus serum on the second day, when in the latter the complete extension and subsequent retrogressive changes preclude any further progress.

The effect of alkali. One part of a m/10 to m/20 NaOH solution added to 20 parts of m/2 or 5/8 m NaCl have also often a beneficial effect on the preservation of the cells and on the outgrowth from the tissue, although the effect is usually not so marked as in optimal solutions of acid, but it may approach this effect. Addition in the same proportion of solutions much stonger than m/10 NaOH has a dissolving effect on the tissue, and solutions much weaker than m/20 NaOH are usually without decided effect, although perhaps there may be a slight temporary influence noticeable from solutions as weak as m/30 or m/50 NaOH. Again as in the case of the acid the amount of alkali present in the solution is diminished through the admixture of some serum or dissolved cell material to the fluid.

Alkali counteracts the dissolving effect of the neutral salt solution and thus preserves the cells. The character of the migrating cells in alkali is similar to that in acid solution; the cells are contracted; a large number of the pseudopods are sharp tongue pseudopods. There exist however some distinct differences between the action of acid and alkali. Alkali usually permits a much larger number of cells to extend and to become dissolved at an early period than acid does. In consequence the field of emigrated cells is in alkali usually thin, while in acid it is densely packed with cells, the more so the nearer the area of growth is to the piece of tissue. In alkali many cells become dissolved, and this applies not only to extended cells, but sometimes even to cells which have not yet fully spread out, and thus there may be found considerable interstices between neighboring cells. These are now free from cells, but cells had been here previous to their solution.

On the second day retrogression has usually become much more marked in alkali than in acid. This combination of the transient preserving effect of alkali added to its tendency to permit after some time the spreading out of well preserved granular cells, may make the picture presented in an alkaline solution not unlike that seen in Limulus serum, where also the cells generally extend in a granular form, the granules remaining preserved for some time following extension. The cells are therefore gradually softening in alkali and thus it comes about that while sharp tongue pseudopodia are quite common in alkaline solutions, broad

and round pseudopods seem to be more frequent here than in an acid solution.

The effect of acid and alkali in hypertonic NaCl solution (3/4 m NaCl solutions were used in these experiments). As in the case of the isotonic NaCl solutions, we found here again variability in the results. In many cases the emigrating or marginal cells hyalinized and cytolysed rapidly and the solution of the tissue led to the formation of an opaque zone and of granular material around the piece. In other cases a zone of contracted granular cells formed around the tissue. In accordance with our former observations the pseudopods were usually found sharp, often threadlike, although other kinds of tongue pseudopodia were also found quite commonly, and sometimes even small drops developed. The average of the pseudopods was however quite distinctly sharper here than in isotonic and hypotonic solutions. Gradually the NaCl solution penetrated into these cells and made them hyaline, especially after they had spread out; but even in contracted cells the hypertonic NaCl solution could enter and cause a solution of the granules. Temporarily the hypertonic solutions preserved the cells in some cases better than isotonic solutions; but whenever a good outgrowth was obtained in NaCl solutions, the zone of outgrowth was greater in isotonic than in hypertonic solutions.

Addition of acid in the same proportion as in the case of isotonic solutions proved in a number of instances beneficial. The area of outgrowth was enlarged and the time during which the outgrowth persisted was increased. In one case we followed the progress of outgrowth over a period of 5 days during which the cells were mostly kept at room temperature. During this whole period the majority of the cells was well preserved, contracted granular, and the pseudopods were on the average very sharp. The extreme sharpness of many pseudopods is characteristic of a combination of hypertonicity and acid reaction. The granuloplasm moves connectedly into the pseudopods in accordance with the great consistency of the protoplasm under these conditions. We find here the sharpest, most threadlike pseudopods, provided the solution permits an emigration of the cells. We find here the same graduation in the strength of the effect of acid as we did in the case of isotonic solutions: we observe all transitions from solutions which fix the cells and thus prevent an outgrowth to optimal solutions in which the consistency of the protoplasm is increased but outgrowth is possible, and to ineffective ones.

Again the optimal solution is obtained through addition of about 10 per cent of a m/100 to m/200 HCl solution. In some cases m/100 HCl was optimal, in others m/200 HCl. In some cases it appeared as if in the case of hypertonic solutions the optimum was at a somewhat greater dilution (m/200 HCl) than in the case of isotonic solutions; but if this should prove to have been more than a coincidence, it cannot apply to all cases, because in some instances m/100 HCl was more favorable than m/200 HCl. It is very probable that with a proper dilution of acid hypertonic solutions will prove to be even more suitable for the long-continued emigration of cell from the piece than isotonic solutions, because in acid hypertonic solution a summation of hypertonicity and increased acidity occurs. Both tend to prevent the taking up of water into the cell, and thus prevent a lowering of the consistency of the cell. But even in hypertonic acid solutions an extension and destruction of cells sets in ultimately. Further experiments will have to decide how long this can be delayed through the selection of suitable concentrations.

In a number of cases no benefit was derived from the use of acid in these solutions. Whether in these cases the hypertonic NaCl solution in itself increased the consistency to such an extent that acid had no chance to act, or whether the proper concentration of the acid had not been found in these cases, cannot be stated at present.

In general even an optimal addition of HCl to a hypertonic solution cannot produce an effect equal to that obtained by the addition of acid to an isotonic solution. But inasmuch as in a hypertonic solution the outgrowth may in certain cases continue over a longer period the outgrowth may come to equal that obtained in isotonic acid solutions at a time when in the isotonic solution the cells had begun to extend and thus to undergo solution processes.

Addition of alkali to hypertonic solutions also produced an improvement in a number of cases; addition of about 10 per cent of a m/10 NaOH solution proved usually better than addition of a m/20 NaOH solution. But again in alkali hyalinization and extension occurred usually earlier than in acid; often on the second day or occasionally even as early as the first day. In alkali drop formation may occur even in hypertonic solutions and in one case circus movements of the drop were observed; furthermore structures resembling eggs with fertilization membranes were found. On the third day the cells in alkaline hypertonic solutions did no longer show active movement.

The effect of acid and alkali in hypotonic NaCl solutions. In m/4 and m/6 solutions of NaCl we found in the majority of experiments instead

of outgrowth an opaque or granular material around the piece, the result of a solution of the margin of the tissue. In some cases, however, an outgrowth occurred such as we obtained in hypotonic NaCl solution quite frequently in previous years. Even in the absence of a definite outgrowth we not rarely found a limited number of cells migrating from the piece; occasionally these cells were suspended in the surrounding fluid. Again we observed the occurrence of the broad tongue and balloon pseudopodia and of transitional forms in hypotonic solutions, in addition to the ordinary tongue pseudopodia which latter were quite numerous. There is evidently a gradation in the character of pseudopods in hypotonic solutions. As long as the cells resist the action of the hypotonic medium, the pseudopods and movements of the granulop'asm may be approximately normal; but when they begin to take up water and thus to diminish their consistency their pseudopods assume the form characteristic of hypotonic solutions and of a lowered consistency. Occasionally even circus movement may be observed in an isolated cell. When the effect of the hypotonic solutions becomes still more pronounced, those structures are produced which we designated as courts and others which we compared with fertilization membranes; still other cells may merely show a swollen appearance and coarse granulations. In such cells the movements of the granuloplasm have usually ceased and the cells are irreversibly injured.

In those cases in which in neutral NaCl solutions a good outgrowth was obtained, it was inferior in hypotonic solutions to that observed in isotonic solutions.

The addition of acid or alkali to the hypotonic solutions produced a distinct and often very marked improvement in many cases, but not in all. It seems that in some cases the cells are not able to resist the effect of the hypotonic solution notwithstanding the counteracting influence of acid or alkali. If we reduce in such cases somewhat the degree of hypotonicity, the acid or alkali may be rendered efficient. For instance, in cases in which a m/4 NaCl was found to be too hypotonic, addition of acid or alkali to a m/3 NaCl solution caused a great improvement in tissues with a low degree of resistance. But in many cases the typical effects of acid and alkali can be produced not only with a m/4 NaCl, but even with a m/6 NaCl solution. Addition of acid rather than of alkali may produce in such cases a remarkable improvement. And yet the extent of outgrowth in an hypotonic acid solution is usually distinctly inferior to that in an acid isotonic solution, but occasionally it may approach it, and in one case even surpassed the growth in isotonic acid

solution on the second day. The distinction due to the osmotic differences are therefore not obliterated by acid or alkali; the effects of the latter are rather added to the osmotic effects. In cases in which there was a good outgrowth in neutral isotonic NaCl solution, the addition of acid and alkali improved markedly the growth in hypotonic solutions and in other experiments in which there was no or only a very slight outgrowth in neutral NaCl solutions, acid or alkali often called forth a very good outgrowth. It is however probable that the number of cases in which addition of acid or alkali was unable to improve noticeably hypotonic solution is greater than the number of experiments in which they were ineffecitve if added to isotonic solutions.

The effect of acid and alkali, when added to hypotonic solutions, in principle is similar to that which we described in the case of isotonic solutions. Especially acid prevents or retards the entrance of water into the cell. It tends to preserve it and to prevent the solution of its granules. Again the cells in the acid solutions are contracted, frequently show the furrow in the posterior end of the cytoplasm; the cells move actively. The pseudopodia tend to be sharp and the more so, the more contracted the cell as a whole is. However, the acid and the hypotonicity of the solution tend to counteract each other, and the acid is not altogether able to prevent a decrease in consistency, and in such cases the pseudopods become broader and balloon-like; occasionally even circus movement may be observed.

While alkali resembles acid in that it tends to preserve the cells and to increase their outgrowth, it is much less effective than acid. As in isotonic media alkali tends to produce a solution of the cells to a much greater extent than the acid. It promotes the extension and subsequent disintegration of cells; but even contracted cells may be dissolved in very hypotonic solutions under the influence of alkali. Thus we are liable to find the fields of outgrowth in hypotonic alkaline solution to be very thin, attenuated. The cells not rarely have the appearance of being fixed, oval or round and flat or somewhat extended.

The combination of marked hypotonicity and alkalinity may in some cases produce changes in cells which are similar to those found in m/4 KCl solutions. Balloons, which may be multiple, and by means of which the cells may move can develop; and in some of these cells circus movements begin to take place. In such cases alkali probably penetrated into the cell in the hypotonic medium and decreased the consistency of the granuloplasm sufficiently to call forth the circus movements. The optimal quantity of acid and alkali in the hypotonic

solutions is not noticeably different from the optimum found for the isotonic solutions; it varies between m/80 and m/200 HCl and m/10 to m/20 NaOH. Lactic and butyric acids also were effective. In some cases it appeared that the stronger acid and the weaker alkali were preferable in hypotonic solutions; but in other cases no such distinction was possible.

The effect of acid and alkali in combination with m/2 and m/4 solutions of LiCl. To judge from the results of a small number of experiments the effect of LiCl in m/2 and m/4 solutions is similar to that of corresponding NaCl solutions. The outgrowth was slight, tongue pseudopodia or sometimes irregular balloons were produced; the cells underwent an early hyalinization. Addition of acid caused an improvement in a number of cases; it increased the amount of outgrowth and caused a greater contraction of the cells similar to that found in NaCl solutions. In several cases m/100 HCl caused too strong an acid effect while m/200 HCl acted favorably; in other cases however m/100 HCl was better. Acid caused an improvement even in hypotonic solutions of m/4 LiCl.

In isotonic solutions alkali preserved the cells for some time and the cells were softer, more regular than in acid solutions; they were either flat or extended and granular; the outgrowth was usually less than in acid, but in a number of cases better than in controls. In isotonic solution m/10 NaOH was optimal, while in hypotonic solutions m/20 NaOH proved to be better.

The effect of the addition of acid and alkali to isotonic (5/8 and 1/2 m) solution of KCl. In many experiments no outgrowth occurred in isotonic solutions of KCl, but instead precipitate or opacity around the piece indicated a solution of the margin of the piece. In other cases a number of cells were freed from the piece as the result of these solution processes. The cells were usually slightly swollen, round, coarsely granular or hyalin; some cells showed courts or fertilization membranes or balloons. Occasionally a small zone of outgrowth occurred; among those cells some had tongue pseudopodia or transitions between tongue pseudopodia and balloons; others showed the typical KCl character.

The movement of granuloplasm in isotonic KCl solutions was usually slight and ceased altogether after some time. The outgrowth in m/2 KCl solutions was relatively best in those experiments in which also in NaCl solutions the cells were found most resistant.

In a number of experiments addition of alkali or hydrochloric, butyric or lactic acid did not produce a distinct improvement. The small number of cells which sometimes were visible around the piece were

coarsely granular; some had courts, others were fixed and did not show any movement; in some cases it seemed that the acid increased the consistency of the protoplasm. In a number of cases acid produced some improvement, which however was marked only in a few instances.

In a case in which some improvement was produced, m/100, m/200 HCl and m/100 butyric acid were about equally beneficial on the first day; on the second day m/200 HCl proved to be better than m/100 HCl. In this case the cells became more contracted under the influence of the acid, the tongue pseudopods increased in number and sharpness; yet despite this improvement there was always some effect of potassium noticeable. Even in the cells in which tongue pseudopodia through which movement took place, had been produced, the protoplasm of the cells was more rigid, rounded off and granular, and instead of the granuloplasm secondarily moving into the pseudopods, the pseudopods were kept in an extended state and pulled the cell as a whole along. Thus the movements were not those of normal amoebocytes, but comparable to the movements of a snail which through its foot pulls the rest of the animal along. Even in specimens in which an improvement was produced through the acid, the potassium effect was still noticeable in the formation of balloon and drop pseudopodia, and even in the most favorable specimens the movements of the granuloplasm were restricted. Occasionally even circus movements occurred in acid KCl solution. There was no or only a very slight progress on the second day. Usually the potassium proved too toxic at that time and the cells were fixed and swollen. Addition of alkali rather emphasized than attenuated the potassium effects and it was therefore not beneficial; acid on the other hand counteracted to some extent the tendency of the cells in K salts to take up water; and thus in acid potassium solutions the cells were usually isolated and rarely formed clumps as they did in alkali.

The effect of addition of acid and alkali to hypotonic (m/3 and m/4) solutions of KCl. In m/4 solutions of KCl a part of the margin of the tissue was often dissolved, and precipitate or an opaque area surrounded the piece. Quite frequently we found a number of isolated cells around the piece and these showed changes characteristic of m/4 KCl solutions. The cells took up water and they became coarsely granular, balloons and typical circus movements developed. A number of cells disintegrated and their disintegration caused the formation of a coarsely granular precipitate. Especially in those experiments in which good results were obtained with NaCl solutions, the effect in the m/4 KCl solutions was likewise better; the number of cells showing circus movements on

the whole being greater, and occasionally more contracted cells with the typical furrows were observed.

Addition of alkali (1 part of m/10 to m/20 NaOH to 10 parts of a m/4 KCl solution) apparently accentuated the potassium effect; the balloons and circus movements seemed to become more frequent; otherwise no marked change was produced. Addition of acid in the usual proportions on the other hand had a tendency to decrease or to prevent the circus movements and balloon formation; although both did occur in acid solution. Frequently the cells were round, hyaline or granular, but in a number of cases tongue pseudopodia or transitions between tongue pseudopodia and balloons were formed, and in accordance with the increase in the number of tongue pseudopodia the number of outgrowing cells was distinctly increased. Again we found that those tissues which also in other solutions showed the greatest activity and resistance gave the best results after the addition of acid. On the whole acid tended to make the cells more consistent, to decrease the taking up of water and the fluidity of the granuloplasm, and thus to counteract the effect of potassium.

When the action of acid was too strong the cells were fixed; if the action was slightly less, the consistency was sufficiently great to permit the formation of tongue pseudopodia, and at the same time it left a few cells sufficiently soft to make possible the circus movements and the formation of balloons.[2]

The effect of isotonic and hypotonic solutions of RbCl and CsCl. In m/2 solutions of RbCl and CsCl the effect is intermediate between that caused by m/2 NaCl and m/2 KCl. The cells emigrate as contracted granular cells with sharp to broad tongue pseudopodia, spread out and become hyaline. In so far they resemble cells in NaCl solution; but at the same time there are noticeable some effects that are similar to those of potassium.

A number of cells are flat and coarsely granular and balloons are noticeable in addition to the tongue pseudopodia. In cases in which the outgrowth is very good in NaCl solutions, the outgrowth in RbCl and CsCl may approach that in NaCl; in such cases the cells are similar to those seen in NaCl solution; they are granular contracted and form tongue pseudopodia. When the outgrowth is very slight or lacking in m/2 NaCl solution, the outgrowth is likewise diminished in RbCl and CsCl, but it is here somewhat better than in NaCl solutions.

[2] In order to make certain that the effect of potassium, which we had described previously, was not due to an admixture, we used a KCl salt prepared by Doctor Doisy which had been specially purified and which he kindly put at our disposal.

In m/4 RbCl and m/4 CsCl the outgrowth was always better than in m/4 KCl and accordingly tongue pseudopodia were somewhat more numerous; and in this respect these solutions resembled m/4 NaCl solutions. But at the same time the formation of balloons and transitions between balloons and tongue pseudopodia was very pronounced and we found distinct circus movement in a number of cases. There were polarized and irregular balloons through which the cells could move. Also coarsely granular, flat cells were found. The greater frequency of balloons and the occasional occurrence of circus movements were effects similar to those observed in m/4 KCl. In m/4 KCl on the other hand the circus movements were more frequent and the outgrowth more limited. In these solutions we have the combined effect of hypotonicity and of specific ion effects on pseudopodia.

RbCl and CsCl are therefore intermediate between KCl and NaCl. On the whole RbCl approaches perhaps KCl more than CsCl, although the difference between RbCl and CsCl is slight.

The effect of acid and alkali in combination with RbCl and CsCl. M/100 and m/200 HCl added to isotonic solutions of CsCl and RbCl in the proportion of 1 to 10 produced a marked improvement. The outgrowth increased and the cells became more contracted, their pseudopods were sharper; but even in these mixtures the effect of Rb and Cs was noticeable and occasionally some balloons and even restricted circus movements ocurred notwithstanding the presence of the acid. RbCl and CsCl counteract therefore partially the effect of the acid. M/10 and m/20 NaOH cause an increased rarefication of the field through solution of cells, a softening of the cells, hypotonic pseudopodia and circus movements. There was thus no improvement noticeable under the influence of alkali.

In hypotonic (m/4) CsCl and RbCl acid added in the usual proportion generally causes an increase in the consistency of the cells; they are made tighter, their pseudopods become sharper. In some cases m/100 HCl was too strong in its action and did not permit outgrowth to take place; in other cases, however, it increased outgrowth. m/200 HCl had a beneficial effect in a number of cases; at the same time it permitted circus movements in a few cases. While generally acid restricts circus movements very much, it does not actually prevent them; and especially in weaker acid they may occur. The Rb and Cs effect is furthermore evident in the formation of balloons even in an acid medium. Alkali again has a softening influence and may promote the development of circus movements; it does not increase the outgrowth.

In a m/2 solution of NH_4Cl the outgrowth was slight; it was still more diminished in a m/4 NH_4Cl solution. The cells in these solutions were usually round or oval, often flat; the granules were well preserved. In extended granular cells a honeycombed appearance was noticeable, perhaps the result of the solution of some granules. Instead of well-formed tongue pseudopodia we find in NH_4Cl solutions short rudimentary pseudopods, which usually are somewhat rounded and often appear as small drops and balloons. Especially common were multiple small drops, which produced the appearance of irregular mulberry cells, not unlike those produced through exposure of the cells to a higher temperature. Some of these cells disintegrated into masses of cell granules; especially in hypotonic solutions. But even in isotonic solutions a number of cells disintegrated and became dissolved. On the whole, however, NH_4Cl had a tendency to preserve the cells and the piece relatively well at least for some time.

Addition of m/100 or m/200 HCl to the NH_4Cl solutions in the usual proportions does not noticeably increase the outgrowth, but it has some of the other typical acid effects. It tends to make the cells more contracted, to produce furrows through contraction of the protoplasm and to increase the number of tongue pseudopodia; yet it cannot overcome the effects of NH_4Cl. Multiple droplets and mulberry cells still appear. In hypotonic NH_4Cl solutions we find flat, round or oval granular cells even in an acid medium.

With alkali (m/10 and m/20 NaOH) no marked effect is produced; the typical mulberry cells are observed, but in some cases the number of tongue pseudopods and the contraction of the cells seem to have increased. Gradually cells extend in these solutions and the extended cells become dissolved.

In solutions of m/2 and m/3 $CaCl_2$ or in solutions slightly less concentrated than m/3 $CaCl_2$ some outgrowth from the piece usually takes place, but the field of growth is generally not dense and often thin. This is due to the fact that in $CaCl_2$ solution the cells readily become hyaline and are soon dissolved; this applies not only to the extended or partly extended cells, but even the contracted cells tend to undergo this change. $CaCl_2$ has furthermore the tendency to keep many cells in a contracted state with sharp pseudopodia. Occasionally the pseudopods are long and rather broad and the cell body small, round, contracted; but some normal, contracted and motile furrow cells were usually observed. In m/3 $CaCl_2$ solution we also found courts and the cells resembling ova with fertilization membranes. On the second day no

progress was usually noted; instead the cell movements had generally ceased.

In calcium chloride the destruction of cells is therefore rather prominent and the viscous material resulting from this process often assumes the form of long radiating fibers which are attached to the piece and extend into the surrounding fluid.

Addition of HCl (1 part m/50 to m/200 HCl to 10 parts $CaCl_2$ solution) does not cause any decided change. The character and fate of the cells is not definitely altered. Sometimes the effect of $CaCl_2$ seems to be accentuated through the addition of acid; at other times it increases perhaps slightly the number of healthy contracted furrow cells. Addition of alkali in the usual proportions does not produce any notable changes.

In solutions of $MgCl_2$ the outgrowth of the tissue varies according to the concentration used. In a 2/9 m $MgCl_2$ solution, the margin of the tissue is dissolved, precipitate is found around the piece and no growth takes place. In a m/3 $MgCl_2$ solution some cells suspended in the fluid are found around the piece. These cells send out tongue pseudopodia and become hyaline after some time. As a result of cytolysis radiating fibers around the piece are produced. In a 5/12 m $MgCl_2$ solution the result is similar, the outgrowth is slight. Contracted granular cells with sharp pseudopodia or flat irregular cells become hyaline at an early period. In a m/2 $MgCl_2$ solution the outgrowth was larger, the cells were better preserved and clumps of granular contracted cells broke away from the piece and moved into the fluid. Subsequently many cells extended and became hyaline; and gradually even granular contracted cells became hyaline. While in the m/2 solution the outgrowth was better than in the other $MgCl_2$ solutions, the cells became hyaline more rapidly than in Limulus serum.

Addition of m/50 or m/200 HCl in the usual proportions to solutions of $MgCl_2$ did at best produce only a slight improvement in the outgrowth. m/80 or m/100 added to m/2 $MgCl_2$ increased perhaps the number of contracted furrow cells with sharp pseudopods; in other cases however the effect of this solution was too strong and merely very contracted granular cells were visible. In 2/9 m $MgCl_2$ solution to which acid had been added some round, granular or spindle shaped cells with tongue or thread pseudopodia were found around the piece. They had probably become detached from the piece through solution processes. No decided improvement was produced through acid in this case.

Solutions of m/2 and 5/8 m $NaNO_3$ behaved somewhat similar to isotonic solutions of NaCl, but in a number of cases the outgrowth was slightly better in the former. The cells were somewhat better preserved in the $NaNO_3$ solutions in experiments in which a NaCl solution proved to be an unfavorable medium. As usual the cells emigrated from the piece as granular contracted cells, they became flat and extended and in this condition the hyalinization or solution of the cells occurred. $NaNO_3$ seemed to make the cells softer and thus to favor their extension.

Addition of m/100 to m/200 HCl in the usual proportion caused in some cases a better preservation of the cells and increased the outgrowth in isotonic solutions of $NaNO_3$; the cells were more contracted and extension and hyalinization delayed; but even under favorable conditions some solution of cells occurred even in acid media in $NaNO_3$ solution. In other cases the acid was either too strong, and led to a hardening and fixation of the cells, or it was too weak and not able to overcome the softening and dissolving effect of $NaNO_3$. m/400 HCl was without effect. It seems that acid is not quite so effective with isotonic $NaNO_3$ solutions as with NaCl solutions. However, it is possible that some accidental variations may be responsible for this difference. A distinct improvement, however, was usually produced in m/2 $NaNO_3$ solutions, through addition of alkali (m/10 or m/20 NaOH). The cells were better preserved, hyalinization delayed and the outgrowth increased; but also in alkali, solution and hyalinization occurred occasionally even in contracted cells.

In m/4 $NaNO_3$ some outgrowth took place, but it was usually slight and the emigrated cells hyalinized after some time. Addition of m/100 to m/200 HCl in the usual proportions produced a very marked improvement. The cells became contracted, the pseudopodia very sharp, the consistency of the protoplasm increased and extension was much delayed. If the solution had a very strong effect and called forth a marked contraction of the cells, the beneficial effect became sometimes clear only on the second day. In alkali some improvement was also obtained, but in the alkaline solutions there was more extension and solution of the cells.

In an hypertonic (3/4 m) solution of $NaNO_3$ the outgrowth was in a number of cases better than in 3/4 m NaCl solution; the cells in hypertonic $NaNO_3$ solution seemed to be somewhat softer than in 3/4 m NaCl. Usually extension and hyalinization and cytolysis followed in this as in the isotonic solution; and in some cases the margin of the piece was dissolved and formed a precipitate.

Addition of m/100 and m/200 HCl led to an improvement which in some cases was very marked; but after some time hyalinization occurred even here. In alkali likewise improvement in outgrowth and preservation took place; but here the process of solution of extended, and even of not extended cells, was more marked than in the case of acid.

The effect of m/2, m/3 and m/4 Na_2SO_4 solutions on the outgrowth and character of cells. In solution of m/2 Na_2SO_4 we usually find a small zone of cells surrounding the piece. The cells are very contracted, round and at first granular; the pseudopods are sharp, often assuming the character of multiple threads. In accordance with this marked contraction and the sharpness of the pseudopods the cells are found to be little sticky and not to agglutinate very readily. At an early period many of these contracted cells become hyaline.

Addition of m/100 to m/400 HCl does not change very markedly the character of the cells; they remain contracted and round and with sharp pseudopods; but the granules are preserved for a longer period of time. The number of cells which surround the piece is much increased; they usually are arranged in two layers, one at the top and the other at the bottom of the drop. The cells are isolated. In a weaker (m/300) (1:10) HCl solution furrow cells of a more normal kind were observed, and some extension took place. Alkali produced at best only a slight improvement. The cells were here also contracted, they became hyaline and many of them were dissolved. In a m/3 to m/4 Na_2SO_4 solution the character of the cells was similar to that found in a m/2 Na_2SO_4. The contracted granular cells soon became hyaline. Addition of m/100 HCl in the usual proportions did not allow a more marked outgrowth, at least on the first day; the solution was too strong. It is however possible that on the second and third day an improvement would have become apparent as the result of the acid.

Addition of m/200 or m/300 HCl produced a very marked improvement. The outgrowth and character of the cells in this solution resembled that in an acid NaCl solution. The hardening effect of the SO_4 ion was to a great extent neutralized; m/10 to m/30 NaOH also produced in several, but not in all, cases an improvement, the cells became more normal in the alkaline solution. Again alkali favored an early extension and hyalinization and solution of the cells after a relatively short period of preservation.

Effect of a solution of Na citrate. In solutions of 2/3 to m/4 Na citrate no definite outgrowth occurs, but a few round, contracted, granular cells with sharp tongue pseudopodia may be visible around the piece.

Addition of m/100 HCl does not cause any improvement. In solutions of 5/12 to 3/12 m Na citrate a few round granular or hyalin contracted cells can be found around the piece whose margin may be partly dissolved.

If 1 part m/2 or 2/3 m Na citrate is added to 9 or 12 parts m/2 NaCl the width of outgrowth, which would occur in the latter solutions, is usually diminished. The cells are very much contracted, round, at first granular, later they become hyalin. The pseudopods are very sharp and often multiple threads. The cells do not readily agglutinate. Added in the same proportions to a m/4 NaCl solution the character of the cells is similar, but in accordance with the lower osmotic pressure of this solution there may be produced some balloon pseudopodia. In combination with m/4 KCl, citrate (added in the same proportions) does not change the character of the cells very much; there occur balloon pseudopods and occasionally even circus movements; neither does citrate distinctly alter the cells in m/2 KCl; perhaps it may increase somewhat the consistency of the protoplasm without noticeably improving the amount of outgrowth. The effect of citrate is thus similar to that of sodium sulphate, but its effectiveness is much greater than that of the sulphate.

In a van't Hoff solution (artificial seawater) no good outgrowth took place. There was usually cytolysis and hyalinization at the margin of the piece. Cells became transformed into radially arranged or arcade like fibers. In three experiments addition of m/100 HCl produced a great improvement; in one of these cases the improvement became definite only on the second day. In three other experiments no improvement was produced.

In sterile seawater (2 experiments) no outgrowth took place; the margin of the piece was dissolved and precipitate formed around it. Addition of m/100 HCl did not call forth any improvement.

In other experiments *Limulus serum was heated to 100°;* the proteid coagulated, and through filtration we obtained a clear fluid. In such filtrate which was free from proteid no or only a slight outgrowth occurred, which was, however, somewhat better than that observed in van't Hoff solution. Addition of acid or alkali in the usual proportions did not lead to a decided improvement.

We see then that a solution whose inorganic composition is similar to that of Limulus serum is much inferior to the latter. This agrees with our previous observations. We found however in experiments carried out in 1920 that occasionally the results obtained in such solu-

tions can approach those in Limulus serum. We find here evidently the same variable factor which we noticed in the case of NaCl solution, and again we must leave it undecided, whether this variability depends upon changes in the resistance of the cells or in a variable admixture of substances extracted from the piece.

In a mixture of 2 cc. filtrate of coagulated Limulus serum and 0.2 cc. of m/10 NaCN cell movement took place similar to that observed in the filtrate alone. The cells sent out pseudopods and showed movements of the granuloplasm. These movements are not prevented by cyanid added in the proportions used in this experiments. A similar result was obtained also in other experiments in which NaCN was added to various salt solutions.

The effect of addition of serum to salt solutions. The difference which we found between the action of salt solutions and of Limulus serum suggested the use of a combination of salt solutions and Limulus serum in various proportions, in order to determine how far addition of salt solution to Limulus serum caused a deterioration of Limulus serum and, on the other hand, how far addition of Limulus serum to the salt solution improved the latter. The mixture of serum and salt solution was in each case heated to above 55° for 30 minutes before use.

1. A mixture of a filtrate of Limulus serum heated to 100° and of fresh Limulus serum in the proportion of 3 parts of the filtrate and 1 part of Limulus serum, or of 3 parts fresh Limulus serum and 1 part filtrate acted almost as favorably as fresh undiluted Limulus serum. The second mixture was perhaps not quite so good as the first one, but it approached it very closely.

2. A mixture of 3 parts sterile seawater + 1 part Limulus serum behaved similar to seawater. Addition of serum did not call forth a marked improvement. In one experiment addition of 1 part of seawater to 3 parts of Limulus serum caused a very marked deterioration of the Limulus serum.

3. A mixture of 3 parts of van't Hoff solution + 1 part of Limulus serum was almost as good as pure Limulus serum.

4. In a mixture of 3 or 5 parts of a m/2 NaCl solution + 1 part of serum there was in the majority of cases no marked improvement noticeable over a m/2 NaCl solution; in some cases an improvement was obtained, although the mixture was in no instance as good as Limulus serum. A mixture of 9 parts of a m/2 NaCl solution + 1 part of serum behaved similarly to a m/2 NaCl solution. In a mixture of 5 parts m/4 NaCl with 1 part of serum only a slight increase in the area

of outgrowth was noticeable; but the combination of the hypotonic NaCl solution with serum led to the production of balloons and occasional circus movements. This combination had therefore an effect not unlike that of a m/4 KCl solution. An addition of 1 part of a m/2 NaCl solution to 3 parts of serum led to a distinct deterioration of the serum; the result however was usually better than in a m/2 NaCl solution.

5. Through addition of 1 part of a m/2 LiCl solution to 3 parts of Limulus serum a distinct deterioration of the Limulus serum was effected, with exception of one case where the result was as good as in Limulus serum; on the other hand, through addition of 1 part serum to 3 or 5 parts of m/2 LiCl some improvement of the LiCl solution was produced, although the mixture did not become the equal of Limulus serum.

6. Addition of 1 part of a m/2 or m/4 KCl solution to 3 parts of serum produced a marked deterioration of the serum. Typical potassium effects appeared. With m/4 KCl solution in the mixture balloons, circus movements and courts were noted; with a m/2 KCl solution in the mixture circus movements were only occasionally found. The serum caused in a number of cells hyaline extension and it also caused perhaps a slight increase in the outgrowth over that found in KCl solution. It seemed as if addition of serum to a m/2 KCl favored in some cases the appearance of circus movements.

The effect was similar if 1 part of serum was added to 3 or 5 parts of a m/2 or m/4 KCl solution; the typical potassium effects were noticeable. There was an increase in circus movements in m/4 KCl solution, and even in m/2 KCl circus movements were found in some specimens. The effect of serum became noticeable perhaps through a slight increase in outgrowth and hyaline extension of cells which was found in these mixtures.

7. Addition of *1 part of serum to 5 parts of a m/2 or m/4 RbCl or CsCl* called forth an increase in outgrowth, which was very marked in some cases; the effect of RbCl and CsCl was still noticeable in the mixture through the presence of occasional balloons and circus movements.

8. Addition of *1 part of serum to 3 or 5 parts of a m/2* NH_4Cl solution did not produce a marked change. The cells characteristic of NH_4Cl (multiple drop pseudopodia or flat extended honeycombed cells) could still be found; the outgrowth was slight. The addition of serum may however become apparent in the hyaline spreading out of cells which

was found in some cases, and also in the greater number of contracted granular cells. Addition of 1 part of a m/2 NH_4 solution to 3 parts of Limulus serum led to a marked deterioration of the serum. Even here the typical mulberry cells of NH_4Cl were visible.

9. Addition of *1 part serum to 3 or 9 parts of a m/2 or m/3 $CaCl_2$ solution* does not produce a marked change; we still find the marked hyalinization and cytolysis of cells characteristic of $CaCl_2$. Addition of 1 part of $CaCl_2$ to 3 parts of serum usually causes a marked deterioration of the serum and an early cytolysis of the cells.

10. Addition of 1 part of a m/3 $MgCl_2$ solution to 3 parts of serum may give results similar to serum or may produce a deterioration of the serum. Mixtures in which the $MgCl_2$ solution predominated over serum were usually much inferior to serum.

We find then that some improvement may be produced through addition of serum to solutions of single salts, but that usually the result is much inferior to that produced through addition of a trace of acid. If about 25 per cent of salt solution is added to Limulus serum a decided deterioration of the serum usually occurs, but in some cases the result may approach that obtained in pure serum. In mixtures of KCl or NH_4Cl and serum the cellular reactions characteristic of these salt solutions are usually present. In case of certain composite salt solutions, like van't Hoff solution, Limulus serum filtrate which resembles Limulus serum, as far as the inorganic constituents are concerned, addition of 25 per cent of Limulus serum makes the mixtures as good or almost as good as Limulus serum. There exists therefore in Limulus serum a certain surplus of proteids, as far as the protective influence of Limulus serum on cells is concerned.

Effect of hypertonicity of salt solutions on the outgrowth of cells. We have seen that in a 3/4 m NaCl solution the outgrowth is not so good as in a m/2 NaCl solution. This could be directly shown in cases in which the tissues grew well in isotonic solutions of NaCl, while in our recent experiments in which in the majority of experiments the cells proved little resistant to a m/2 NaCl solution, the difference between hypertonic and isotonic solutions became apparent after addition of acid. We found, however, that in the tissues which showed little resistance in 3/4 m and m/2 NaCl solutions, some improvement could be produced through the use of a concentration intermediate between m/2 and 3/4 m. A moderate increase in osmotic pressure seemed to produce an improvement. Solutions intermediate between a 3/4 and 5/8 m solution were usually found best. It is probable that the slightly hyper-

tonic solutions retarded the entrance of fluid into the cells and thus exerted a preserving effect. The improvement thus produced was however only moderate and not comparable to that called forth through addition of acid to isotonic NaCl solutions under optimal conditions.

DISCUSSION

Our results indicate that at the isoelectric point amoebocytes are least resistant to injurious influences and have the greatest degree of permeability to the surrounding fluid. We see that acid and to some extent alkali may protect the cells not only in isotonic but in certain cases also in hypotonic and in hypertonic solutions. Under the influence of the acid in optimal concentration the cells, their consistency, the character of pseudopods, the movement of granuloplasm remain healthy. This relation between consistency of protoplasm and character of pseudopods and amoeboid movement is in agreement with our previous conclusions (11).

We may therefore assume that acid prevents the surrounding fluid, salts as well as water, from entering the cells. In hypertonic acid solution an increase in consistency occurs and here we find an increase in the average sharpness of pseudopods. While acid may not prevent the withdrawal of fluid from the cells in hypertonic solutions, it protects to some extent the cells against the entrance and effect of injurious constituents of the surrounding medium. The same protective influence is noticeable to a much greater degree in isotonic and hypotonic solutions. It is very probable that acid acts mainly on the outer layer of the cell; but at present we cannot exclude the possibility that it may in some cases in addition exert a similar effect on inner boundaries between colloid particles and the surrounding liquid. In addition to this protective influence both acid and alkali exert specific effects, acid having an hardening and alkali a softening effect on the cells.

In accordance with this increase in consistency caused by acid pseudopods are on the average sharper in acid than in neutral solutions. The softening effect of alkali leads to a more rapid spreading out and destruction of the cells. The beneficial influence of alkali is therefore usually much more transient than that of acid. The improvement caused by acid or alkali is however limited by the effect of the other substances in the solution. In media which cause such marked alteration of the cells, as we find in solutions of KCl, NH_4Cl and certain other substances, acid and alkali show no or only a very slight beneficial effect.

The results we obtained with organic acids indicate that the effects observed in acid solution do not altogether depend on changes in pH concentration, but that the anion may also play a certain part. We find then that in both acid and alkaline solutions the amoebocytes may be active.

Limulus and spider crab blood show according to the measurements of Dr. S. Morgulis a pH of 7.4 to 7.6, a value which does not differ very much from that found in mammalian blood serum.

Within the body and under normal conditions the amoebocytes are therefore suspended in a slightly alkaline medium, and they are, as we have previously seen, on the whole non-motile. It is due to two factors that the cells remain well preserved in this medium: *1*, the action of the proteids of the blood; and *2*, the lack of those stimuli which are active in the changed environment. The normal lining of the body spaces leaves the amoebocytes unchanged; they remain flat, elliptic, non-sticky discs. Some organic constituents of the blood, in all probability the proteids, protect the cells; this follows from the marked difference which is usually found in Limulus serum with its proteids intact and in Limulus serum filtrate obtained from coagulated blood serum or in a van't Hoff solution. The blood proteids protect therefore in all probability the cells for some time against those injurious changes which would otherwise take place at or near the isoelectrical point.

The real pH concentration of the media which surrounded the cells in our experiments remains still to be determined. Substances extracted from the piece of tissue undoubtedly neutralize a part of the acid. So far we have made only a preliminary test with an indicator of the reaction of the fluid which surrounded the piece and which had been made acid through addition of m/100 HCl in the usual proportions. It was still found acid at the end of the experiment. A good outgrowth had taken place in this case.

There may be in addition to the direct effect of acid and alkali an indirect way in which they may perhaps exert a beneficial action. By combining with the proteid material in the fluid protein salts are probably formed which may bind a certain amount of water which would thus be prevented from acting on the blood cells.

It is probable that different kinds of cells and tissues differ in their sensitiveness to acid; it is furthermore certain that a greater concentration of neutral salts counteracts certain injurious effects of acids. Those cells are presumably better able to withstand injurious effects of acid which are better adapted to higher osmotic pressures of the surrounding

medium. The earlier investigations of Michaelis and Kramsztyk (12) and the recent studies of Schade, Neukirch and Halpert (13) make it certain that also in mammalians many cells are adapted to a higher hydrogen ion concentration than is normally found in the blood.

In the fluid surrounding the tissues and in the tissues themselves variations occur in the concentration of acid which are caused through the metabolism of the cells. An increase in the acidity of tissues has been held to be the principal cause of edema. From our experiments we may conclude that if a certain degree of hydrogen ion concentration is reached in the fluid directly surrounding the cells the entrance of water into the tissues proper may be prevented. Such an increase in acidity would therefore have rather a preventive than a promoting effect on edema of the tissues in contradistinction to the tissue spaces.

It is probable that what holds good in the case of amoebocytes applies equally in the case of certain other labile cells; in this connection we must however take into consideration differences in the adaptation to osmotic pressure on the part of different kinds of cells; this might modify the effect of acid and alkali on these cells and tissues.

Our experiments very clearly bring out one of the factors which may cause the death of tissue cells. We have previously shown that many mammalian tissue cells have a potential immortality; they die because of unfavorable conditions which develop in their environment.

An analysis of the conditions which lead to the death of cells in the body, and of the factors which tend to preserve the cells, would be of great importance. We know that a low temperature which diminishes metabolism prolongs the life of cells and organisms. In the case of tissues this preserving influence has been used in many instances; but it has also been shown by Jacques Loeb and Northrop (14) and Raymond Pearl (15) to hold good in the case of whole organisms (Drosophila). Here the duration of life is, as these authors have shown, a function of the intensity of the metabolism and therefore of the temperature. In the eggs of Arbacia Jacques Loeb could increase the resistance of the cells to injurious influences through withdrawal of oxygen (16).

In the case of the blood cells of Limulus we recognized another factor as injurious and causing processes of dissolution, namely, the spreading out of the cells in response to stereotropic stimuli (10). A cell which in the contracted state might be preserved even in a not fully adequate medium, becomes dissolved as soon as it begins to spread out, or even with increased intensity, after it has become fully extended. If we pour

very gently on amoebocytes, which are firmly or loosely fixed on the surface of a slide, a fluid which has a moderately injurious influence on the cells, we notice that it is always the extended cells which are dissolved first, while the contracted cells resist much longer the injurious effect of the solution. We may then conclude that in all probability the process of spreading out leads to an increase in the permeability of the cells which now become more readily accessible to the injurious effect of water and other constituents of the surrounding medium which tend to enter the cells. By delaying the spreading out of the cells through various means, for instance, through placing the tissue on a base of vaseline, or paraffin, or through increasing the consistency of the cells through a combination of salts with acid or alkali, or perhaps through a slight increase in the osmotic pressure of the surrounding medium, we may be able to delay the spreading out of the cells and thus in favorable cases to prolong the life of the cells over a considerable period of time.

In this connection we wish to point out that the stereotropic spreading out of cells is a phenomenon of considerable importance in tissue formation in general, and we must consider the possibility that an increase in the permeability, which we found associated with the process of spreading out in the case of amoebocytes, may under similar conditions also occur in other cells and perhaps play a part in tissue differentiation.

SUMMARY AND CONCLUSIONS

1. The effect of various salt solutions on the cells emigrating from cultures of experimental amoebocyte tissue accords with the effect of these solutions, formerly described by us, on blood cells suspended in a drop of blood which has been received directly in these solutions. We found a decrease in consistency of the cells under the influence of potassium salts, a production of pseudopods followed by hyalinization and cytolysis in sodium chloride, an intermediate condition in RbCl and CsCl, a preservation of granules and the formation of mulberry cells in NH_4Cl, a marked hyalinization and cytolysis in $CaCl_2$, a contraction and increase in consistency of cells in Na_2SO_4 and citrate solutions. In accordance with our former investigations we found that all those solutions which tend to decrease the consistency of the cells cause a broadening and rounding off of pseudopods (hypotonic solutions, salts of K, Rb, Cs). While an increase in consistency causes the pseudopods to be finer and sharper (hypertonic solutions, sulphates, citrate); in NH_4Cl multiple rudimentary droplet pseudopodia may appear.

Through removal of the proteids from the blood serum a deterioration is caused in the Limulus serum; addition of blood serum to the filtrate of coagulated Limulus serum in proportions of 1 part of serum to 3 parts of filtrate or van't Hoff solution restores the efficiency of the latter as a medium for the blood cells. Addition of blood serum to solution of single salts, which are in themselves injurious for the blood cells, may produce an improvement, without however producing an effect equal to that seen in pure blood serum. Addition of as little as 10 per cent serum to the salt solution is without marked effect.

We found a considerable variability in the effect of solution of NaCl on cells emigrating from amoebocyte tissue; while in some cases the outgrowth was as good as in Limulus serum, in others a rapid hyalinization and cytolysis occurred. This effect of NaCl solutions on the amoebocyte tissue would be in accordance with the direct effect of NaCl on isolated amoebocytes. It remains to be determined whether this variability in the effect of NaCl solution on cells emigrating from amoebocyte tissue is due to variations in the resistance of the amoebocytes in different animals, or, to an unequal admixture of substances extracted from the piece of tissue.

2. Addition of acid and alkali in certain proportions causes not only a marked improvement in the outgrowth of the cells, provided the injury caused by the salt solutions to which acid or alkali are added has not exceeded a certain limit, but the outgrowth in acid solutions may definitely surpass that in Limulus serum, which otherwise represents the most favorable medium for the amoebocytes of Limulus. Near the isoelectric point solutions seem to be most injurious as far as the preservation, as well as the activity, of the amoebocytes is concerned. The beneficial effect of acid and alkali may be noticeable even in hyper- and hypotonic solutions. Acid and alkali probably decrease the permeability of the cells, or of certain constituents of the cells, and thus save the cells from the injurious effect of water and substances dissolved in the water, which otherwise would change the colloids of the cells. Especially acid increases the contraction and consistency of the cells and accordingly also the sharpness of the pseudopods.

3. Both organic and inorganic acids are effective in concentrations which do not differ very much; it is therefore probable that the effect of the acid is not altogether due to changes in hydrogen ion concentrations which it produces.

4. In addition to the preserving action common to acid and alkali, both exert specific effects on the amoebocytes, in alkali the extension

and subsequent solution of cells taking place much more rapidly than in acid; the former have a softening, the latter a hardening effect upon the cells and their membranes.

5. Certain organic constituents of the Limulus blood serum, probably its proteids, exert a protective influence upon the amoebocytes; this constituent, however, is not so effective as acid in optimal concentration.

6. The stereotropic extension of the amoebocytes leads to degenerative changes in these cells, probably as the result of an increase in the permeability caused by the process of spreading out. Conditions which counteract this spreading out of the cells tend therefore to prolong their life for a considerable period of time, provided these conditions are not otherwise injurious. The spreading out of amoebocytes, which we observe in vitro, is analogous to the extension of somatic cells of higher organisms during embryonic development. In both cases the extension leads to the production of tissue-like structures.

We wish to express our thanks to Miss E. M. Paxson for her assistance in these experiments.

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STUDIES ON THE PHYSIOLOGY OF GASTRO-ENTEROSTOMY

I. The Regurgitation of Intestinal Contents in Normal Dogs and Dogs with Posterior Gastro-enterostomy

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Different investigators have in the past few years reported finding duodenal juices in the normal stomach, especially when the acidity there was of fair degree. Boldyreff (1) studied the circumstances by which duodenal contents might be expected to be found in the stomach. He found that this reflux took place when the acidity of the stomach was higher than the duodenal mucosa could tolerate, a mechanism which tended to keep the acidity of the stomach at a low level. He called this mechanism the self-regulation of the acidity of the stomach contents. His work was done on dogs with gastric fistula. Morse (2) found that regurgitation often occurred in anesthetized pithed dogs when the acidity reached 0.2 per cent and nearly always with acid of higher concentration. He also found that the higher the concentration (0.1 to 0.5 per cent) of acid the greater the regurgitation. The rate of emptying of the stomach decreased with the increase of acidity. Hicks and Visher (3) introduced by means of a tube 150 cc. of 0.5 per cent HCl into the stomach of dogs and noted a regurgitation in fifteen minutes in 30 per cent of the tests and in thirty minutes in 45 per cent. They state that the introduction of 0.4 per cent HCl in a human subject showed no regurgitation at the end of twenty minutes. A psychic secretion averaging 32.6 cc. (acidity 0.411 per cent) obtained in an individual with a gastric fistula caused a reflux in 40 per cent of ten trials. Rehfuss and Hawk (4) assert that the introduction of 0.5 per cent HCl in a human subject caused regurgitation of the alkaline intestinal secretion. Moppert (5) reported spontaneous reflux of bile in 72 per cent of cases examined using the Einhorn thread. Jarno (6) found regurgitation in the human stomach after introduction of relatively weak acid solution, 0.08 per cent.

We thought it worth while to repeat Boldyreff's experiments on normal dogs, using a stomach tube for introducing the acid and aspirating samples and then to make a similar study of these animals after gastro-enterostomy to learn the effect on the normal mechanism of gastro-enterostomy. We had no difficulty whatsoever in using the stomach tube. After the first few times the tube was introduced the dogs showed no signs of discomfort and did not struggle. We were surprised to see the ease with which the test could be carried out.

Dogs of average size and in good condition were chosen. They were kept in the laboratory during the period of normal test but were given exercise outside every other day. They received no food for twenty-four hours previous to the test but were allowed water at all times. One hundred to 150 cc. of 0.5 per cent HCl were given with a stomach tube, the dog was then allowed to get on his feet and walk about for a minute or two, giving opportunity for the acid introduced to mix with the stomach contents. A sample was then obtained by aspiration and titrated. The presence of water, if any was in the stomach, was shown by this titration. A second sample was taken fifteen minutes after introduction of the acid, another in thirty minutes, etc., until the acidity was reduced to 0.1 to 0.2 per cent, or the stomach empty. The bile coloration was used as an indication of a reflux from the duodenum.

Our results on the normal dogs confirm the findings of Boldyreff. The average length of time for reduction of the acidity to 0.1 to 0.2 per cent was in our experiments seventy-five minutes to ninety minutes. The first appearance of bile varied considerably with different dogs and different experiments. It was sometimes present in fifteen minutes, often in thirty minutes and a few times was delayed for an hour.

Paterson (7) demonstrated the presence of bile in the gastric contents by Gmelin's reaction in 73 per cent of his patients after gastro-jejunostomy. He also stated that the total acidity was reduced 30 per cent, partly due to the neutralization of the acidity by the bile and pancreatic secretion and partly to earlier stimulation of the pancreas bringing about an earlier diminution of the gastric secretion (8). Lemon (9) found a reduction of the acidity after gastro-jejunostomy of 39 per cent total and 46 per cent free. A study of two hundred cases was made and the reduction ascribed to flowing into the stomach of the alkaline secretion of the duodenum.

The gastro-enterostomy opening in our dogs was made $2\frac{1}{2}$ to 3 cm. long on the posterior wall of the antrum of the stomach near the pylorus. The opening in the duodenum was made at the tail of the pancreas.

Care was exercised in feeding for a few weeks to permit complete healing about the stoma. In no case were there untoward symptoms following the operation. All the animals made a speedy recovery and soon regained any loss of weight. Allowing two or three weeks for recovery the tests were again made as before the operation. Bile was often present in the first sample, the one taken almost at once after the acid was given. It was regularly present after fifteen minutes and the process of partial neutralization was carried on with corresponding rapidity. After thirty to forty-five minutes the acid was reduced to 0.1 to 0.15 per cent and the stomach nearly empty.

Whether or not after gastro-enterostomy the acid brings about an earlier stimulation of the pancreatic secretory mechanism or the mechanism for regurgitation is rendered more efficient, that is, the stoma permits a freer flow of intestinal juices into the stomach, we are unable to say. Cannon (10) has shown that the stoma may act as a valve, letting contents pass into the stomach but closed in the other direction, especially when the stomach is distended. Since there was a shorter emptying period in our experiments after gastro-enterostomy it must be accounted for in one of two ways. Either the earlier neutralization allowed passage sooner through the pylorus or a part of the contents passed through the gastro-enterostomy opening. These questions are being investigated.

The manner in which the duodenum forces back its contents as reported by Boldyreff (1) is by antiperistalsis. Hicks and Visher (3), however, claim not to have seen any antiperstaltic movements in their experiments but rather constriction bands. This point will be reported on with further studies in this field.

SUMMARY

1. The alkaline juices of the duodenum are regularly regurgitated into the stomach of the dog within thirty to forty-five minutes after the introduction of 100 to 150 cc. of 0.5 per cent HCl.

2. After introduction of 100 to 150 cc. of 0.5 per cent HCl, the acidity of the stomach contents is reduced to 0.1 to 0.15 per cent in seventy-five to ninety minutes and the stomach empties at a rate in proportion to the rate of regurgitation of duodenal juice.

3. In dogs with posterior gastro-enterostomy duodenal regurgitation takes place within fifteen minutes after introduction of 100 to 150 cc. of 0.5 per cent HCl and the acidity of the stomach contents is reduced to 0.1 to 0.15 per cent in thirty to forty-five minutes.

Experiments on a number of animals gave concordant results. Typical experiments on two animals before and after posterior gastro-enterostomy are shown in the table.

TABLE 1

150 cc. of 0.5 per cent HCl introduced

TIME INTERVALS	NORMAL		AFTER GASTRO-ENTEROSTOMY	
	Total acidity per cent	Character of specimen	Total acidity per cent	Character of specimen
		Dog 7		
minutes				
2	0.4466	Clear	0.4922	Turbid
15	0.4102	Clear	0.2918	Bile ++
15	0.3646	Bile +	0.2553	Bile ++
15	0.3554	Bile +	0.1824	Bile +++
15	0.3007	Bile ++	0.1185	Bile +++
15	0.1824	Bile +++		
		Dog 4		
2	0.4375	Clear	0.4740	Bile, trace
15	0.3646	Bile, trace	0.3098	Bile ++
15	0.3281	Bile, trace	0.1454	Bile +++
15	0.2644	Bile ++	0.06	Bile ++++
15	0.2553	Bile ++		
15	0.1915	Bile ++		

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THE PULSE IN THE PORTAL VEIN[1]

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The portal vein occupies a unique position in the anatomy of mammals, lying, as it does, between two great sets of capillaries, those of the splanchnic region on the one hand and those of the liver on the other. Therefore the inflow of blood into the portal system must be controlled to a large extent by the caliber of the splanchnic vessels, while the outflow must likewise be controlled by the caliber of the liver vessels. Many conditions might disturb the equilibrium of this double-ended regulation as, for instance, variable vasomotor activity, changes in heart rate and blood pressure, or even changes in vena cava pressure. Since resulting variations in blood flow must react on certain activities of the liver cells, the importance of the portal circulation becomes at once apparent.

This importance combined with the fascination of the unusual regulatory mechanism of the portal blood flow, especially in its relation to liver volume, has attracted many investigators, but no one appears to have inquired into the nature or importance of the pulse variations taking place during each cardiac cycle. Indeed it might well be suspected that such a pulsation does not exist, inasmuch as the pulse wave tends to be abolished completely in its passage through the splanchnic vessels. Such, however, is not the case, for we found in our experiments on dogs a cardiac portal pulse of definite contour.

By recording this cardiac pulse graphically and by studying the changes in its contour under various conditions, a considerable knowledge was obtained of the portal mechanism—how, at times, the outflow of blood exceeded the inflow until equilibrium became established while, at other times, just the opposite occurred and inflow exceeded outflow.

Anatomy of the portal vein. A diagram of the portal system of the dog may be found in an article by Burton-Opitz (1). The main trunk is

[1] This investigation was aided by a grant from the American Association for the Advancement of Science.

formed by the union of the superior and inferior mesenteric veins. About 2 cm. cephalad from this juncture, the vein is joined by the gastro-splenic branch. The portal vein in the dog is 4 to 6 cm. long and 6 to 8 mm. in diameter when distended with blood.

Method. Dogs of medium weight were carefully anesthetized with morphine and chloretone, and throughout the experiments were permitted to breathe normally. Through the gastro-splenic branch a small curved cannula 27 mm. long and 2 mm. in diameter was introduced into the portal vein, the open end of this cannula being turned toward the splanchnic capillaries. The distance from the mouth of the cannula to the capillaries forming the inferior mesenteric vein (capillaries of the large intestine) averaged 8 cm. while the distance from the cannula to the capillaries forming the superior mesenteric vein (capillaries of the small intestine) was about 20 cm. On the insertion of the cannula the gastro-splenic vein had to be tied, thereby causing congestion of the spleen and a small portion of the stomach. Although within the lumen of the portal vein, the cannula, because of its small diameter, interfered but little with the passage of portal blood.

The cannula was rigidly connected to a Wiggers' optical manometer (2) covered by light rubber dam, in order to make it sensitive. The cannula of a similar manometer, equipped with a heavier rubber membrane was inserted into the abdominal aorta at the forking of the iliacs. Heart sounds were recorded simultaneously. A tambour, held to the shaved chest of the animal by an elastic belt was connected by rubber tubing to a Wiggers' heart sound segment capsule (3). A tuning fork vibrating fifty times a second gave the time measure.

Thus accurate and simultaneous records of the heart sounds, abdominal aortic pulse, and portal pulse were taken.

The contour of the portal pulse. The pressure variations in the portal vein really represent changes in the volume of blood contained in that vein with certain modifications introduced by extra-portal mechanical causes. Figure 1 shows a typical record of the portal pulse taken simultaneously with records of heart sounds and abdominal aortic pulsations. The heart rate in this record was 182 per minute, a rate not unusually rapid in the dog, so we may consider this record as showing the normal portal pulse of the dog.

In general, the curve falls during systole and rises to a rounded summit during diastole. The systolic fall, however, is interrupted by a sharp peak which occurs almost simultaneously with the peak of the abdominal aortic pulsation and this is followed by a sharp decline.

With greater detail, we may describe the curve as follows: At the exact instant that systole begins (as indicated by the large vibration of the first heart sound) the curve has already begun to decline gradually. This gradual decline, however, is interrupted by a sharp peak, *a-b*, after which it falls abruptly to a trough, *c*. Then the upstroke of the main wave, *d*, commences. The beginning of this rise precedes slightly the early vibrations of the second heart sound; in other words, it begins in late systole and is continued well into diastole so that the crest of the

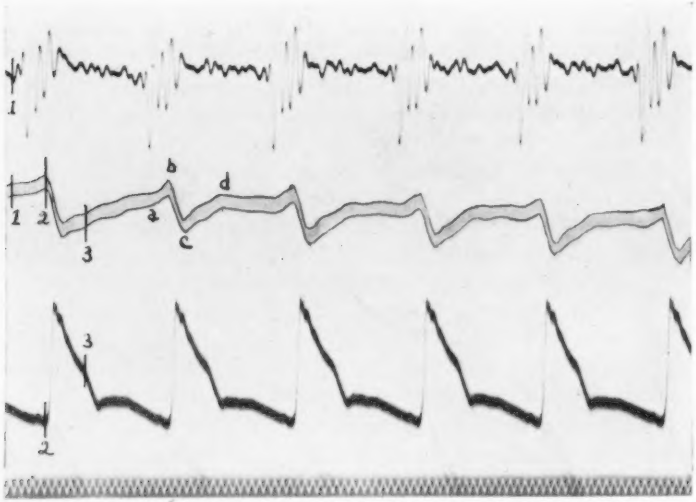

Fig. 1. Simultaneous records of apex sounds, portal pulse, and abdominal aortic pulse. Time relations: *1*, beginning of systole of heart; *2*, time that ejection wave of arterial pulse appears in abdominal aorta; *3*, end of systole waves *a*, *b*, *c* and *d* referred to in text.

curve is approximately mid-diastolic. From the crest, the curve declines slowly until the next systole occurs and the cycle is repeated. It should be pointed out that neither the amplitude nor the duration of the systolic peak, *a-b*, was always as pronounced as in this illustration. In some animals the rise *a-b* was barely indicated, but in all cases the sharp depression, *b-c*, was clearly shown. In some of our records no respiratory variations of pressure curves were found; in others, however, cardiac pulsations were superimposed on more gradual variations synchronous with respiration. These changes caused by respiration

consist in a considerable fall in the level of the entire curve at inspiration and a relatively steep rise during expiration. The contour of the wave seen in the cardiac portal pulse remains approximately constant during this respiratory fall and rise in general pressure. In normal curves a level stretch follows the fall in pressure.

It is difficult to determine the exact reason for these respiratory variations. Their abruptness and close relation to phases of inspiration and expiration suggests a mechanical cause, possibly a movement of the abdominal viscera due to the descent of the diaphragm rather than a direct effect of varying intrathoracic pressures on the filling and emptying of the portal system.

The effect of slowed heart-rate. The human heart is only about one-half as rapid as that of the dog. To more nearly approach the actual condition in man, therefore, it became necessary to study the portal pulse when the dog's heart rate was slowed sufficiently to lie within human range. To attain this end, the right vagus was sectioned and the peripheral end stimulated by a mild faradic current.

With such stimulation the general level of the portal curve falls rather abruptly (fig. 2). If the diastolic pause is greatly prolonged, the curve drops to its minimum level in one beat. If the diastolic periods are not lengthened so much, this fall in portal pressure takes place over several beats. With maintenance of vagal stimulation, the curve continues to be inscribed at its lowest level. This change of general pressure level due to the slowed heart indicates that the minute volume entering the portal system is reduced more than the efflux out of the liver. With removal of vagus stimulation the curve rises to its normal level in steplike fashion during several beats.

On casual examination, the contour of the cardiac portal pulse appears to change from normal when the heart is slowed (fig. 3). The most obvious difference is that the summit of the *d* wave is followed by a more prolonged diastolic fall not present in rapid rates normal to our dogs. The entire wave consequently appears of larger amplitude, its summit being either rounded or flat. Time relations show, however, that the summit *d* occurs approximately at the same time after the end of systole. The fact that it is no longer in mid-diastole is entirely due to the greater prolongation of diastole which gives opportunity for a subsequent diastolic fall to express itself more clearly.

Effect of intravenous saline infusion. Inasmuch as the arterial blood pressure was somewhat reduced due to unavoidable manipulation of the intestine, it was questionable whether our so-called normal curves

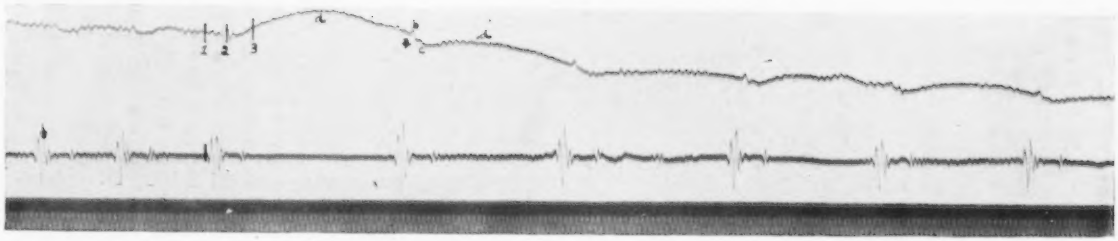

Fig. 2. Record of portal pressure and heart sounds showing cardiac slowing causes reduction of general level of portal pressure and an increasing pressure of the *d*-wave. (Very much reduced in reproduction.)

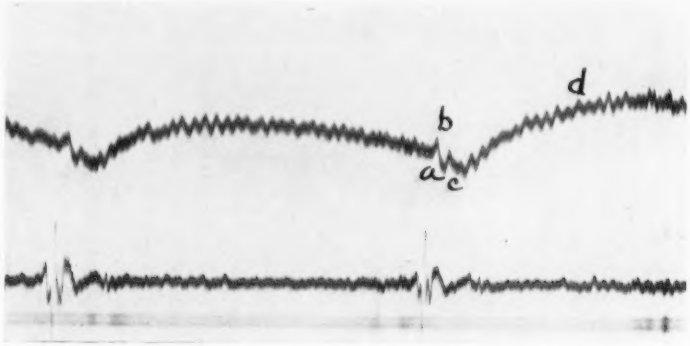

Fig. 3. Portal pressure waves during vagal slowing (less reduction).

really represented the true normal after all. We therefore determined to maintain an effective systolic discharge of the heart by means of saline infusion in an effort to ward off the changes of circulation inaugurated by the pre-shock state. Normal salt solution was therefore introduced slowly into the right jugular vein in quantities varying from 600 to 1000 cc. per dog. As the general level of the portal pressure rises during each infusion, the details of the cardiac pulsations become more distinct. As shown in figure 4, the fall during systole is more abrupt, broken as usual by an unchanged peak, *b*. The diastolic ascent is steeper and the summit, *d*, higher. As compared with the normal rather flat curve, the wave under infusion appears distinctly rounded. From the details so far analyzed, we may estimate approximately the contour of the portal pulse in unoperated animals with slow and rapid hearts. In man, we believe it will closely resemble the contour of a dog's portal pulse under saline infusion and slowed heart rate.

Interpretation. In endeavoring to interpret the cardiac variations in portal pressure, it is important to bear in mind that the portal pressure at any moment is determined by the balance between the inflow of blood through the splanchnic capillaries and the outflow through the liver capillaries. Whenever the portal pressure is on the increase, inflow exceeds outflow and vice versa whenever the portal pressure declines, outflow exceeds inflow.

With every systolic increase of pressure in the tributaries of the mesenteric arteries a larger pulse volume is sent through their capillaries into the portal vein. Owing to the relatively great capillary resistance the entrance of this volume pulse into the portal system is somewhat delayed. This accounts, we believe, for the elevation of pressure late in systole and its continued elevation in early diastole (*c-d*, fig. 4). Occasionally, as in figure 1, a stable equalized condition obtains for a short while, accounting for the flattened summit of *d*. Then follows a state where outflow exceeds inflow, due, no doubt, to the fact that the portal vein receives a smaller quantity of blood as the arterial pressure falls during diastole. Consequently, the portal pressure then falls definitely during the remaining portion of diastole. If the heart is rapid, diastole is short and the next systole supervenes before this fall has had time to express itself. Consequently, as in figure 1, the pressure continues to rise almost throughout diastole and falls during systole. Were these the only factors determining portal pressure variations during systole and diastole, we should expect the curve to show an unbroken fall during early systole. Actually we find a small peak, *a-b*, followed by a sharp

drop, *b-c*, at the beginning of systole. As shown in actual records, the elevation *a-b* occurs 0.04 to 0.06 of a second after the first sound, while the depression, *b-c*, begins slightly before or with the rise of pressure in the abdominal aorta at its bifurcation. In the interpretation of these sharp variations, *a*, *b* and *c*, it is difficult to conceive of any sudden change in outflow or inflow sufficiently rapid to cause this acute change in pressure. It might be suggested that there is blocking of the portal outflow at the juncture of the portal and hepatic tributaries by higher pressure in the hepatic artery. If this were the case, however, we should expect an increase of pressure throughout systole and not a sharp decrease following the sudden rise. Furthermore the rise, *a-b*, apparently occurs

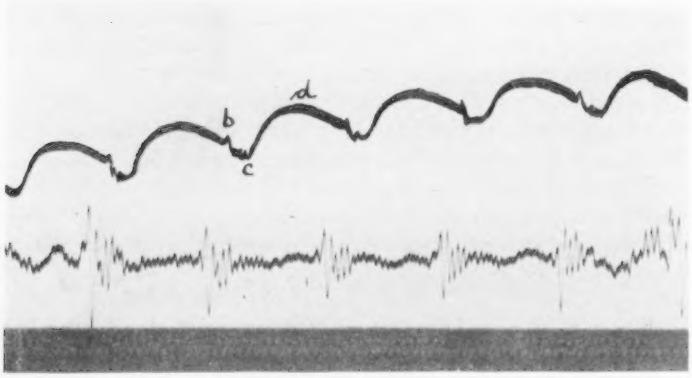

Fig. 4. Portal pressure curves (rapid heart) and heart sounds during moderate saline infusion, showing augmentation of the main wave, *d*.

before the pulse in the abdominal arteries, and precisely when such an effect may be looked for, viz., at point *b* the portal pressure drops, i.e., changes in a direction the reverse of that anticipated. It is also conceivable that the peak and subsequent fall is similar to the arterial impact recorded in jugular tracings, or that it is caused by the transmitted pulsation of the heart through the diaphragm. After mature consideration of all these and other possibilities in relation to the time of their occurrence, we believe that the rise and fall of the *a-b-c* wave may be most satisfactorily explained as due to an impact and traction effect of the following kind: Since the rise *a-b* often occurs before the ejection phase has started, and as it varies in amplitude in different animals, we interpret this as a mechanical impact given by the change in cardiac

position transmitted through the diaphragm and liver to the portal system. Since the sharp decrease *b-c* occurs practically simultaneously with the aortic pulsation, it appears that abdominal arterial pulsations in some way exert a slight traction effect on the portal system, and produce this sharp decrease in pressure. The close proximity of many large arteries to the portal vein makes such an explanation probable.

TABLE 1

B-C INTERVAL IN SECONDS		HEART RATE PER MINUTE		PRESSURE CHANGE
Normal	Peripheral vagus stimulation	Normal	Peripheral vagus stimulation	
0.06	0.06	192	96	−
0.06	0.07	182	59	−
0.02	0.02	240	83	−
0.025	0.025	240	150	−
0.02	0.03	207	115	−
0.052	0.054	208	54	−
0.07	0.07	142	66	−
0.052	0.058	186	72	−
0.06	0.07	186	53	−
0.056	0.58	192	102	−
0.034	0.038	144	87	−
Normal	Central vagus stimulation	Normal	Central vagus stimulation	
0.04	0.06	186	180	−
0.02	0.036	222	222	−
0.06	0.09	142	124	−
0.05	0.062	186	182	−
Normal	Epinephrin injection	Normal	Epinephrin injection	
0.052	0.07	139	68	−
0.058	0.078	146	153	
0.034	0.04	186	168	−
Normal	Amyl nitrite inhalation	Normal	Amyl nitrite inhalation	
0.054	0.04	139	150	+
0.04	0.02	184	196	+
Normal	After saline infusion	Normal	After saline infusion	
0.052	0.056	208	216	
0.058	0.058	158	130	

If the peak, *b*, represents the beginning of an extraneous effect due to an arterial pulsation and the rise at *c* indicates the arrival of the systolic volume pulse through the splanchnic capillaries, then the time required for the volume pulse to travel through the mesenteric arteries and their

capillaries can be calculated by this interval. Such calculations of this *b-c* interval have shown that point *c*—the beginning of the rise of the diastolic wave—is from 0.02 to 0.07 of a second after *b* depending undoubtedly on the size of the animal and on circulatory conditions in the splanchnic vessels. It may be seen, however, from the tabulated results that this interval is rarely less than 0.04 of a second and, furthermore, that it is not affected by changes in systemic blood pressure such as are produced by cardiac slowing, nor was it materially affected by such volumes of saline as were infused during our experiment.

Evidence favoring our interpretation of curves. A substantiation of our interpretation of the portal pulse waves is found in experiments in which the resistance of the splanchnic capillaries was experimentally altered. If the splanchnic arterioles are contracted in any way, we should expect a greater delay in the transfer of a pulse volume into the portal veins, i.e., the *b-c* interval should lengthen. Furthermore, portal inflow being

Fig. 5. Portal pulse curve and heart sounds during action of epinephrin. Note practical abolition of all portal pulse waves except small variations, *a-b*; *b-c* interval increased, *d*-wave entirely absent.

diminished and outflow continuing, we should expect the entire *d* wave to be of smaller amplitude. If, on the other hand, the splanchnic arterioles are dilated, the reverse effects viz., a shortening of the *b-c* interval and an increased amplitude of the d-wave, would be expected.

An increased splanchnic resistance was brought about by central vagus stimulation. With no change in heart rate, the general level of the portal pressure curve declined, thus demonstrating the reduced minute volume of blood reaching the portal vein. Similar but more intense vasoconstriction was also produced by experiments in which epinephrin was injected. Again there was a prompt and profound fall in the general portal pressure. Under moderate vasoconstriction thus produced, the wave *d* became much smaller while during intense epinephrin constriction, it disappeared entirely (fig. 5). As the epinephrin effect wore off this wave very gradually reappeared. While the chief wave, *d* in the portal pulse disappeared under profound epinephrin

effect, the wave *a-b* caused by the mechanical impact of the heart persisted, unaltered in contour. With these variations in contours, the anticipated effects on the duration of the *b-c* interval were found. As shown in the table, both after reflex vasoconstriction induced by central vagus stimulation and after splanchnic constriction from epinephrin, the *b-c* interval is materially lengthened. Contrary effects were observed in experiments in which the splanchnic vessels were dilated by inhalation of amyl nitrite. The general pressure curve then increased. The *d* wave increased in amplitude and, as shown in the table, the *b-c* interval was definitely shortened.

SUMMARY

1. A portal pulse consisting of two waves is produced in the portal vein during each cardiac cycle.

2. The first wave, *b*, we interpret as due to extraneous impact and traction, the second or real wave, *d*, we believe due to the changing balance which occurs between inflow and outflow of the portal system, at different times of the cardiac cycle.

3. Our interpretation of the d-wave is as follows: Following each systolic ejection a certain pulse volume reaches the splanchnic vessels. Owing to the splanchnic resistance there is a delay of 0.02 to 0.07 of a second (average 0.04 of a second) in the transference of this increased volume to the portal vein. Consequently, the portal pressure begins to rise later than the aortic pulse by this interval. This transfer of blood does not cease with the end of systole but continues into diastole, consequently, portal pressure reaches its maximum relatively early in diastole. At this stage, the splanchnic inflow exactly equals hepatic outflow and wave *d* has reached its summit. Thereafter outflow exceeds inflow and portal pressure declines.

4. The amplitude of the chief or *d* wave is determined by *1*, the length of diastole; *2*, by the pulse volume entering the portal vein. The latter is determined by *a*, the systolic discharge of the left ventricle; and *b*, the degree of splanchnic constriction or dilation.

5. Evidence is submitted that the rise of the *b* wave is probably due to a transmitted cardiac impact while the sharp drop is attributed to an arterial traction.

6. According to this interpretation of the *b* and *c* waves, the *b-c* interval represents the time required for the systolic pulse volume to be transferred from the splanchnic tributaries to the portal vein. This interval is unaffected by changes in heart rate or moderate saline infu-

sion but is decreased whenever the splanchnic vessels are constricted, and increased whenever they are dilated.

The direction and guidance of Prof. Carl J. Wiggers in this investigation is gratefully acknowledged.

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BLOOD PRESSURE RESPONSES TO HYPERSYSTOLIC COMPRESSION OF TISSUES

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The present work was begun as a study of the effect of smoking on blood pressure. This involved an attempt to follow qualitatively blood pressure variations continuously over considerable periods and to record these graphically with special apparatus. It was during the course of these observations that several phenomena presented themselves, an investigation of which forms the basis of the work presented here.

Apparatus and principle. We may first describe the apparatus and methods employed in the original problem, i.e., the effect of smoking on blood pressure. Airtight aluminum jackets covering the hand and about two-thirds of the forearm were cast from plaster molds. To make the jackets airtight they were impregnated with paraffin and removable rubber gaskets were laid along the approximating flanged edges of the two halves, which were held tightly together by bolts. A rubber cuff fastened to the proximal end of the jacket made a tight junction between jacket and upper forearm. Air pressure was applied in the jackets and piston recorders were connected with them through an Erlanger sphygmoscope to record the oscillations of the pressure.

The principle that a rise in blood pressure manifests itself by an increase in the amplitude of pulsations transmitted through a cuff set in the neighborhood of systolic pressure and by a decrease in the amplitude of pulsations transmitted through a cuff set in the neighborhood of diastolic pressure, the reverse occurring in connection with a fall of blood pressure (1), (2), was employed. This method has been in use in this laboratory for the past five years; work on its quantitative standardization is now in progress. As used here it reveals only qualitative changes, i.e., whether the blood pressure rises or falls, but does not give quantitative results. Jackets were used instead of cuffs with the

idea that they would be much less painful when the compression was to be maintained for some time, and this supposition was verified by experience. Only a slight local discomfort was experienced during a compression lasting 40 to 60 minutes. The term "hypersystolic jacket" will be used in reference to the jacket by which the compression above systolic blood pressure was applied; "hypodiastolic jacket" for the one by which compression slightly below diastolic was applied.

Method. The method of procedure in the first set of experiments was to take the subject's blood pressure by the auscultatory and graphic methods, the Erlanger instrument being used. The pressure in one jacket was then set about 10 mm. Hg. above systolic and that in the other at about or a little below diastolic. After a few minutes the subject began to smoke a cigar. Mild cigars were used and were smoked in a leisurely manner. The smoking was usually continued until one cigar had been smoked, about 25 to 30 minutes. The compression was maintained and pulsations recorded for some time, 10 to 20 minutes, after smoking was discontinuted. During the course of the record the blood pressure was taken at about 5-minute intervals by the auscultatory and graphic methods, thus giving a quantitative check on our continuous record. Both smokers and non-smokers served as subjects. Eleven experiments were conducted but the data of three of them, experiments 1, 2 and 4, are not presented because we failed to take graphic and auscultatory blood pressure determinations along with the continuous record; therefore no quantitative blood pressure figures can be given in these. However, we may state that the same changes in the amplitude of oscillations were shown in them as in those presented.

Results. In every case a rise in both systolic and diastolic pressures was obtained. This is manifested, *1*, by an increase in the amplitude of the oscillations from the jacket set above systolic pressure; *2*, by a decrease in amplitude of the oscillations from the jacket set below diastolic pressure, and *3*, by the findings of the auscultatory and graphic blood pressure determinations. There was also usually some nausea and sometimes weakness. These symptoms appeared after 25 to 30 minutes of smoking or 30 to 35 minutes of compression in the jackets, this being soon after the blood pressure began to rise. They appeared with equal frequency in smokers and non-smokers; in fact, the most severe reaction, with a marked late fall in blood pressure and almost complete collapse, was in a regular smoker (exper. 7).

Experiment 7. Subject H. L., smoker.

Time in minutes

0 Blood pressure 106/70, pulse rate 71. Compressing pressure in jacket, 113 mm. Hg. Average amplitude of oscillations, 4.2 mm.

21 Blood pressure 130/86, pulse rate 98, average amplitude of oscillations, 5 mm.

31 Blood pressure 130/92, pulse rate 94, slight vertigo, smoking stopped, blood pressure begins to fall.

42 Blood pressure 100/68, pulse rate 68, subject weak, pale, sweating.

46 Blood pressure 92/60, pulse rate 59, pulsation oscillations barely visible, subject weak and cold. Pressure in jacket released, blood pressure at this time 88/60. Immediately on release of compression subject feels better.

52 Blood pressure 110/70, subject feels normal.

Another type of reaction is shown in experiment 5.

Experiment 5. Subject W. B. H., non-smoker.

Time in minutes

0 Blood pressure 105/70, compression jackets set at 114 and 67 mm. Hg., average amplitude from hypersystolic jacket 2.6 mm., from hypodiastolic jacket 10.5 mm.

33 Blood pressure 128/90, average amplitude from hypersystolic jacket 4.2 mm., from hypodiastolic jacket 4.4 mm., subject begins to feel weak.[1]

38 Blood pressure 105/85, average amplitude from hypersytolic jacket 2.8 mm., from hypodiastolic jacket 5.6 mm., subject sick and weak. Smoking stopped, compression continued.

42 Blood pressure 110/92, average amplitude from hypersytolic jacket 2.6 mm., from hypodiastolic jacket 2.8 mm., subject almost collapsed. Decompressed.

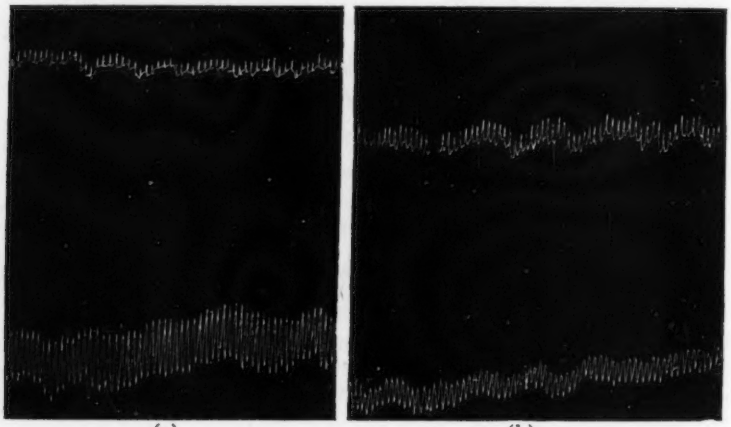
57 Blood pressure 112/82, subject feels normal. Sections of the record of this experiment are shown in figure 1.

The result obtained in experiment 5 is in contrast with the preceding case where the blood pressure fell to subnormal during the compression. Why such severe symptoms should occur with a fall of blood pressure to its original level is not clear; they may probably be at least in part

[1] In these first experiments we were interested in the effect of smoking on blood pressure and our notes dealt with the time relations of the blood pressure changes to the smoking rather than to the compression, because at that time we did not realize that the compression was the factor bringing about the blood pressure changes. Since in all cases, however, the compression was applied about 5 to 8 minutes before smoking started, the period of compression can be ascertained from the latter; thus in the case at hand the blood pressure was 128/90 about 33 minutes after onset of compression, i.e., 26 minutes after beginning smoking.

explained by the effect of the smoking itself. This secondary fall of blood pressure while the compression is still being maintained is not constant in all the experiments. It occurred only in the smoking experiments and in by no means all of these. It is presumably due to a vasomotor fatigue, probably aggravated by the nicotine.

These results, a marked rise in blood pressure, followed in some cases by a fall and the accompanying unpleasant symptoms, are quite at variance with the findings of previous observers who have studied the effect of smoking on blood pressure (3), (4) and who find in non-smokers



(a) (b)

Fig. 1. Tracings of oscillations from jackets *a*, immediately after application of compression, and *b*, about 30 minutes later. Blood pressure at time of *a* was 105/70; at time of *b*, 128/90. Reversal of amplitudes of oscillations from hypersystolic and hypodiastolic jackets is well shown.

on smoking one or two cigars a rise of 10 to 20 mm. Hg. in systolic pressure, followed by a fall when the toxic symptoms usual in non-smokers appear, in moderate smokers a gradual rise of 8 to 10 mm. and in "excessive" smokers no change or a rise of 2 to 4 mm. It will be noted that our blood pressure changes are much greater than these, furthermore they occur in habitual smokers to the same extent as in non-smokers. The data of a typical experiment with a habitual smoker are given in table 1.

A control experiment was now run, i.e., compression applied to the subject's hands and forearms while he was not smoking.

Control 5. Subject K. A. M., non-smoker.

Time in minutes

0 Blood pressure 110/72, average amplitude from hypersystolic jacket 4.1 mm., from hypodiastolic jacket 3.4 mm., pulse rate 74. Compressing pressure in jackets 116 and 67 mm. Hg.

28 Blood pressure 120/78, average hypersystolic amplitude 4.5 mm., average hypodiastolic amplitude 3.1 mm., pulse rate 68.

35 Blood pressure 140/90, average hypersystolic amplitude 9.6 mm., hypodiastolic amplitude 3.1 mm., pulse rate 62.

40 Blood pressure 144/90, average hypersystolic amplitude 11.6 mm., hypodiastolic amplitude 3.2 mm., pulse rate 59.[2] Now decompressed. No markedly unpleasant symptoms, as with a combination of compression plus smoking. There was, however, some sensation of heat, throbbing in the head and some sweating.

40½ Blood pressure 135/76.

41 Blood pressure 130/76.

43 Blood pressure 120/72.

46 112/70.

49 112/70. Amplitude of oscillations could of course not be followed after decompression.

It thus became obvious that some factor other than the smoking was influencing the blood pressure. Accordingly this continuous graphic method of following blood pressure was dispensed with and the effect of blood pressure on smoking was followed by making blood pressure determinations at frequent intervals by the auscultatory and graphic methods, using the Erlanger instrument. The results of these observations have no further concern with this paper, except that it may be stated here that no significant blood pressure changes were found to result from smoking.

Leaving aside, then, the question of the effect of smoking on blood pressure, and passing on to a consideration of the effects of the compression, further controls without smoking were run, using the apparatus diagrammed in figure 2. Here only one jacket was used and oscillations from it were not recorded, the jacket serving merely as a compression

[2] It is of course recognized that the pressure in the hypersystolic jacket is no longer hypersystolic after such a rise in systolic blood pressure, but the changes in the amplitude of oscillations occur with the pressure in the cuff or jacket in the neighborhood of systolic; in case of such a rise as the one under consideration the increased pulse volume also contributes to the amplitude of oscillations; but it may be noted that the decrease in the amplitude of oscillations from the hypodiastolic jacket is occurring in spite of the tendency of the increased pulse volume to increase the amplitude.

chamber. The pressure in the jacket was set well above systolic and was recorded by a writing lever on the manometer float, *A*. The manometer, *B*, on the Erlanger sphygmomanometer was also made to record on the kymograph. Thus, after obtaining zero points, one could apply the pressure in the sphygmomanometer cuff and carry out graphic determinations by the continuous decompression method without watching to get the manometer reading; one can go back and measure the records at leisure. Furthermore, the subjective error due to reaction time is eliminated. Auscultatory blood pressure determinations were

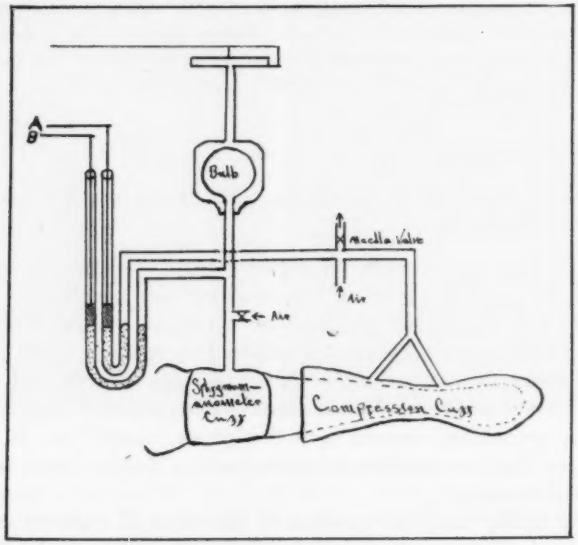


Fig. 2. Diagram of apparatus used in control experiments.

made on the opposite arm. The arrangement will be apparent on examination of the diagram of apparatus, figure 2, and of a typical record reproduced as figure 3. The results of one such control, sections of the record of which are shown in figure 3, may be given in some detail here.

Subject K. A. M. Initial blood pressure 114/75 by auscultatory, 113/76 by graphic. In every case the auscultatory figure is the average of two consecutive determinations which, except during the period of blood pressure waves, always checked within 2 mm. The corresponding graphic determination was made as

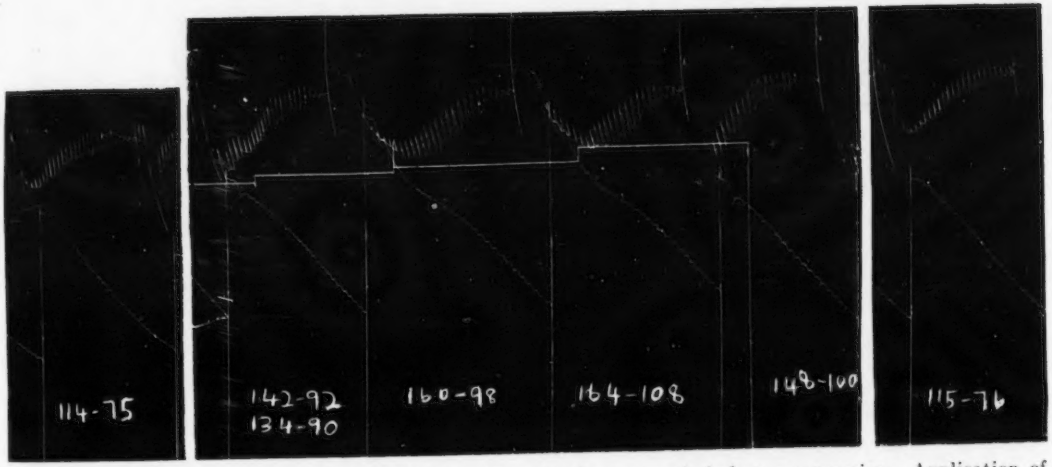


Fig. 3. Sections of record made in control 1. First section, normal, before compression. Application of compression in sphygmomanometer cuff records as vertical line, decompression as an oblique falling line. Actual blood pressure values by the graphic method can be measured by laying off the horizontal distance from the arc on tracing of oscillations to the oscillations presenting the systolic and diastolic criteria, respectively, then finding the level at which the falling pressure line is this horizontal distance from the vertical line. The height of this level above the base line, multiplied by four, since figure is reduced one-half, gives the blood pressure. Corresponding determinations by auscultatory method are written in the figure. In the second and fourth sections blood pressure waves are seen; e.g., at beginning of tracing the systolic pressure is above the cuff pressure, in a few pulses it falls below cuff pressure although the latter has itself fallen in the meantime; as the cuff pressure continues to fall it soon falls again below the systolic. The compressing pressure in the jacket is recorded as the horizontal line just beneath the oscillation tracings. It is seen to be released in section five. The last section shows a tracing made after blood pressure had returned to normal.

soon as possible, within a few seconds, after the auscultatory, and the auscultatory figure was written on the record under its corresponsing graphic determination as soon as it was taken. Compression of 150 mm. Hg. applied at 3:50 p.m. This compression was kept practically constant during most of the experiment, slight fluctuations due to variations in source of air pressure occurring, but pressure was always within a few millimeters of 150 and even the slight variations

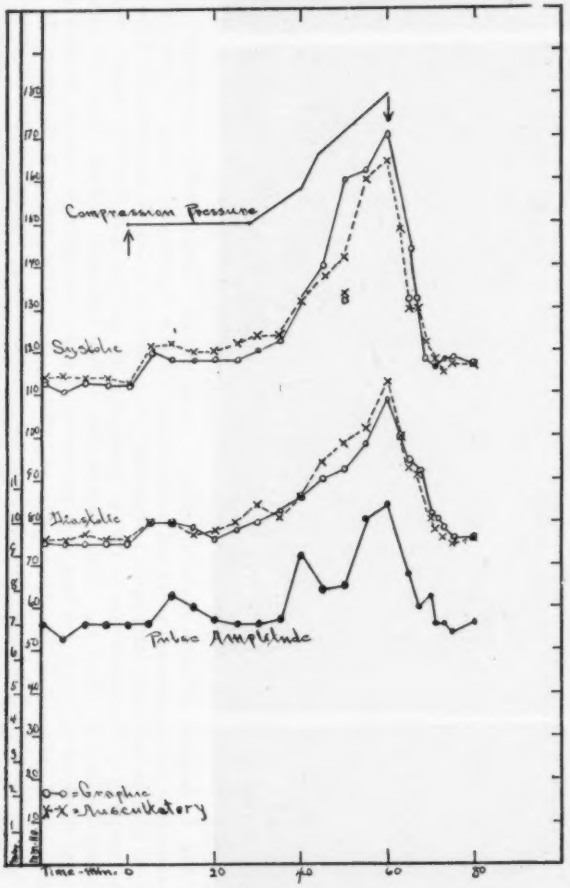


Fig. 4. Left vertical column of figures designates millimeters amplitude of oscillations in sphygmomanometer tracings. Right vertical column designates millimeters Hg. of blood pressure and compressing pressure.

were of brief duration. Toward the end of the experiment, as the blood pressure rose, the compressing pressure was gradually raised so as to exceed it slightly and was 180 mm. Hg. at the close of the experiment. Immediately upon application of compression blood pressure rose to 122/80 auscultatory, 120/80 graphic. At 4:16 blood pressure 124/80 auscultatory, 120/84 graphic. Shortly after this waves of blood pressure appeared. At 4:40 blood pressure was 136/90 auscultatory, 140/95 graphic. From this time on, at least until the highest pressures were established, there were marked waves of blood pressure; consecutive auscultatory determinations made at as short an interval as possible might show a difference of 20 mm. in the systolic reading. The same phenomenon can be seen in sections 2 and 4 of figure 3, which is made from the record of this experiment, the change in form in these being already present at the highest pressure shown, then disappearing and reappearing several pulses later on in the record. At 4:45 blood pressure was 160/98 auscultatory, 162/102 graphic. At this time subject complained of feeling hot, had a severe headache and was sweating profusely. Shortly after this the blood pressure was 164/108 auscultatory, 170/112 graphic. The arm was then decompressed. Three minutes later blood pressure was 148/100, auscultatory and graphic; 5 minutes later 130/94 auscultatory, 132/92 to 144?/92 graphic. Thirteen minutes after decompression blood pressure was 115/76 auscultatory and 118/79 graphic, subject felt normal. These results, as well as the amplitude of oscillations from the Erlanger instrument, are seen in graphic form in figure 4 and are tabulated in table 2. It will be seen that the amplitude of oscillations increases with the arterial pressure.

The symptoms in the control are not so severe as in the smoking experiments, although the blood pressure in some of the controls was run up higher than in some of the smoking experiments. The absence of nausea in the controls is the most striking difference. Undoubtedly the action of the tobacco superimposed upon the greatly disturbed circulatory condition is a factor in the production of symptoms but it is evident that the circulatory changes alone can produce definite symptoms. It will be noted that the rise in blood pressure begins earlier, within 20 to 25 minutes after compression starts, in the smoking experiments than in the controls, 35 to 45 minutes.

Tables 1 and 2 give the full data of one smoking and one control experiment, respectively. It is found impracticable to tabulate all the data of all the experiments. Tables 3 and 4 have therefore been constructed which give a summary of the data of all the eight smoking and five control experiments, respectively.[3]

Further investigation of the factor or factors bringing about the marked rise in blood pressure just noted seemed desirable. It could be

[3] N. S. and S. in tables 3 and 4, refer to non-smokers and smokers respectively. G and A, after blood pressure figures, refer to graphic and auscultatory methods, respectively.

ascribed neither to the tobacco nor to the sucking action of the smoking, as both of these were eliminated as factors in the controls. The explanation which first suggested itself was that the rise in blood pressure was a

TABLE 1

Data of a smoking experiment, no. 9. Habitual smoker.

	TIME	COMPRESSING PRESSURE IN "HYPERSYSTOLIC" AND "HYPODIASTOLIC" JACKETS	BLOOD PRESSURE, GRAPHIC AND AUSCULTATORY	MAXIMUM, MINIMUM AND AVERAGE OSCILLATIONS FROM "HYPERSYSTOLIC" JACKET	MAXIMUM, MINIMUM AND AVERAGE OSCILLATIONS FROM "HYPODIASTOLIC" JACKET	PULSE RATE PER MINUTE
	minutes	*mm. Hg.*	*mm. Hg.*	*mm.*	*mm.*	
Before compression			105–70			
			105–70			
	5	113–67	105–70 G	3.1	4.8	65
			105–70 A	1.8	3.0	
				2.5	3.8	
	10	113–67	107–72 A	3.6	3.3	73
				1.6	2.3	
				2.5	3.1	
After application of compression	24	113–67	120–80 G	8.5	3.8	71
				4.2	2.8	
				6.0	3.2	
	35	113–67	145–95 A	12.4	3.7	68
				6.8	2.4	
				9.2	2.8	
	41	113–67	142–92 G	14.5	3.4	
				9.4	2.4	68
				12.2	2.8	
After release of compression	1	137–85 G				
	6	120–78 G				
	9	112–74 G				
	14	110–78 G				

response to the local ischemia produced by the compression exerted on the arm in the jackets. It might be thought of as a compensatory effort to force blood into the tissues rendered anemic by the compression. Such a response is of course well known in the case of increased intra-

TABLE 2

Data of control 1. Non-smoker

	TIME	COMPRESSION PRESSURE	BLOOD PRESSURE, GRAPHIC AND AUSCULTATORY	AMPLITUDE OF MAXIMUM OSCILLATION
	minutes	*mm. Hg.*	*mm. Hg.*	*mm.*
Before compression			113–76 G 114–75 A	7.0
			111–76 G 115–75 A	6.5
			113–78 G 114–75 A	7.0
			112–76 G 114–75 A	7.0
			113–77 G 112–75 A	7.0
After application of compression	2	150	120–80 G 122–80 A	7.0
	6	150	118–80 G 122–80 A	7.8
	10	150	118–76 G 120–78 A	7.5
	15	150	118–78 G 120–76 A	7.2
	21	150	118–80 G 122–78 A	7.0
	26	150	120–84 G 124–80 A	7.0
	32	150	122–81 G 124–82 A	7.2
	40	150	132–86 G 132–85 A	9.0
	50	150	140–95 G 136–90 A	8.0

TABLE 2—*Concluded*

	TIME	COMPRESSION PRESSURE	BLOOD PRESSURE, GRAPHIC AND AUSCULTATORY	AMPLITUDE OF MAXIMUM OSCILLATION
	minutes	*mm. Hg.*	*mm. Hg.*	*mm.*
After application of compression			160–100 G 134–100 G	8.2
	52	150	142–92 A 134–90 A	
	55	170	162–102 G 160–98 A	10.2
	60	180	170–112 G 164–108 A	10.5
After release of compression	3		148–100 G 148–100 A	8.5
	5		132–92 G 144?–92 G 130–94 A	7.5
	7		132–90 G 130–92 A	7.8
	9		118–83 G	6.8
	9		122–84 A	
	11		116–82 G 118–78 A	6.8
	13		118–79 G 115–76 A	6.5
	15		118–76 G 116–75 A	6.5
	20		116–76 G 116–76 A	6.7

cranial pressure but so far as we knew at that time no such observations had ever been made upon extracranial tissue. Moreover, the conditions in the two cases are not strictly comparable, since in the case of increased intracranial pressure the vasoconstrictor center itself is pre-

TABLE 3

Summary of data of smoking experiments

EXPERIMENT NUMBER	INITIAL BLOOD PRESSURE	PRESSURE IN HYPERSYSTOLIC JACKET	HIGHEST BLOOD PRESSURE ATTAINED	TIME, AFTER COMPRESSION, OF APPEARANCE OF RISE OF BLOOD PRESSURE
	mm. Hg.	*mm. Hg.*	*mm. Hg.*	*minutes*
3 NS	108–74 A	118	122–84 A	17
5 NS	105–70 G 107–72 A	114	128–90 G 128–90 A	18
6 S	98–70	108	118–84	30
7 S	106–70	113	130–92	15
8 S	105–70	113	116–85	28
9 S	105–70 G 105–70 A	113	145–95 A	20
10 S	108–80 G	116	118–98	34
11 S	104–62 G 106–62 A	112	138–80	24

TABLE 4

Summary of data of control experiments

EXPERIMENT NUMBER	INITIAL BLOOD PRESSURE	COMPRESSING PRESSURE	HIGHEST BLOOD PRESSURE ATTAINED	TIME, AFTER COMPRESSION, OF APPEARANCE OF RISE OF BLOOD PRESSURE
	mm. Hg.	*mm. Hg.*	*mm. Hg.*	*minutes*
1 NS	112–76 G 114–75 A	150	170–112 G	40
2 S	108–70 G	135	134–80 G	?
3 NS	112–72 G	135	144–85 G	40
4 NS	112–74 A	158	144–100 A	44
5 NS	110–72 G 112–72 A	116	144–90 A	23

sumably rendered ischemic and thereby stimulated, while in the case at hand the local ischemia must bring about the rise in blood pressure either reflexly, chemically or in some other way than by direct stimulation of the vasomotor center such as obtains in the case of increased intracranial pressure.

Accordingly the pressure was set in the jacket at different levels in order to determine at what level a rise in blood pressure would be produced. It was found that there was practically no change in blood pressure until the pressure in the jacket was raised above the systolic pressure. Also, there was not an exact correspondence between the height of the pressure in the jacket and the extent to which the blood pressure would be raised, but in every case except two, controls 1 and 4, the systolic blood pressure rose to a height greater than that in the jacket, up to 165 mm. Hg. In one of these exceptions, control 4, the pressure was rising rapidly when the compression was released and there is no reason to believe that it would not have exceeded the compressing pressure if the latter had been kept on a short time longer. In the other exception, control 1, the blood pressure was not given a chance to rise above the compressing pressure, for as the former rose the latter was purposely raised to keep it above the former. Thus at the close of the experiment conducted in this way the compressing pressure was 180 mm. Hg., the systolic blood pressure 170 mm. Hg. This failure of exact correspondence may be due to variations in the relation of arteries to the bony framework in different individuals, requiring greater excess above systolic to render one person's forearm ischemic than is required for another. The fact that under a compression 10 mm. Hg. above systolic the systolic pressure might rise 30 to 40 mm. indicates, as is of course well known, that the circulation is still seriously interfered with even though the compressing pressure may no longer exceed the systolic pressure. It will also be noted that in one and the same individual the time required for the development of the rise of pressure varies from 25 to 45 minutes.

An effort was next made to determine the mechanism acting to produce this rise of blood pressure. The pressure in one jacket was set 27 mm. above systolic while the other jacket was used as a plethysmograph. If the rise in blood pressure were due to a vasoconstriction we should expect a diminution in the volume of the plethysmographed arm coincident with the rise in blood pressure. It was found, however, that the volume of the plethysmographed arm *increased* during the rise in blood pressure. This increase was maintained during the period of

heightened blood pressure. The arm volume receded rapidly when the blood pressure fell on release of the pressure on the other arm. Some of the details of this experiment are here given.

Plethysmograph tracing with compression.

Time in minutes

0 Blood pressure 108/70. Pressure in compression jacket 135 mm. During first half-hour no significant change in plethysmograph tracing or in blood pressure.

30 Blood pressure 116/74.

35 Blood pressure 122/78. Plethysmograph curve starts up rather rapidly, steady upward trend.

40 Blood pressure 134/76. Plethysmograph curve going slowly but steadily upward. Arm now decompressed. Plethysmograph tracing at once assumes a consistent downward trend, blood pressure at same time falling.

44 Blood pressure 116/74, plethysmograph tracing coming down steadily.

48 Blood pressure 114/76, plethysmograph tracing coming down consistently with a gradual retardation.

It seemed, then, that we had shown that the blood pressure rises to overcome a local ischemia produced by compression of extracranial tissue and that this rise is not accompanied by vasoconstriction in a plethysmographed arm. Possibilities for further investigation on the human subject as to the nature of this response seemed practically exhausted. Accordingly we turned to animal experiments for further light. Naturally, one of the first questions which presented itself was whether or not we had in our observations a clue to the explanation of one type of clinical hypertension. We might conceive of scar tissue in the renal capsule contracting and subjecting the kidney to a pressure great enough to interfere with renal circulation, the response being a rise in blood pressure. Or local areas of kidney tissue might be thus affected by interstitial scarring, or an analogous condition might obtain in other organs. Such a conception is of course not original but so far as we knew no one had subjected this conception to an experimental test.

A compression chamber was therefore devised by which pressure could be applied to the kidney without occluding the renal vessels or ureter. It consisted of two airtight bladders, one to be placed on each side of the kidney, and a brass case to surround these. This was made in two halves which could be quickly taken apart. The vessels and ureter came out through openings cut into the approximating edges of the halves. The case itself was not airtight, the air pressure being

applied inside the bladders, the case acting merely as a guard for the bladders. Precautions were taken in applying the chamber not to injure the renal nerve supply passing through the pelvis.

In the first experiment a rabbit was used. Chloral and urethane were administered by stomach tube one half-hour before experiment started and were followed by light ether anesthesia. Cannula in right carotid. Compression chamber placed on left kidney through lumbar incision. Initial blood pressure 50 mm. Hg. Pressure to which kidney was subjected and blood pressure were recorded on kymograph, same base line being used, so that relation of pressure on kidney to blood pressure could be seen at a glance. Animal in only fair condition. After several minutes of constant blood pressure a pressure of 60 mm. Hg., about 10 mm. above blood pressure, was put on the kidney. No change in blood pressure resulted. This was repeated several times, the pressure being raised as high as 100 mm. Hg. and the duration of the pressure varying from 30 seconds to 5 minutes. In no case was any significant change in blood pressure observed. At the conclusion of the experiment the pressure was raised to 70 mm. Hg. and the renal vessels occluded by a clip. Kidney then decompressed and chamber removed. Kidney seen to be blanched. Clip then removed from vessels, when kidney immediately became red and was distinctly seen to increase in volume; at the same time slight hemorrhage started from the small capsular vessels which had been torn in removing the pericapsular fat while putting on the compression chamber. This seemed to be good evidence that the kidney had really been rendered anemic by compression.

The experiment was repeated with a dog, using ether anesthesia. The same result, i.e., no significant change in blood pressure, was obtained. Another experiment with a decerebrate cat as subject also was negative.

It seemed possible that the rise in blood pressure obtained in man might be due to the discomfort produced by the compression. In order to investigate this point a human subject, K. A. M., was given "twilight," the morphine-scopolamine anesthesia regularly used on the obstetrical service in Barnes Hospital. An initial subcutaneous injection of morphine, grain $\frac{1}{6}$ and scopolamine, $\frac{1}{133}$, followed in 45 minutes and 90 minutes by scopolamine, grain $\frac{1}{133}$, was given. At the end of 90 minutes the subject was in the peculiar state which has been clearly described by H. Schwarz (5). In the case at hand the subject was quite unconscious although he stirred restlessly at times. At the conclusion of the experiment he had no recollection of what had happened. He did not recover consciousness sufficiently to walk unassisted until 3 hours after the conclusion of the experiment. The results of this experiment were the same as those with the conscious subjects, i.e., a marked rise of blood pressure, the systolic rising from 115 to 155 and the diastolic from 75 to 85 mm. The pressure in the jacket was set at 130 mm. Hg., 15 mm. above the initial systolic. Owing to the inability of the

subject to coöperate it was found impracticable to record the pulsations but blood pressure determinations were made at frequent intervals with both graphic and auscultatory methods. The rise in blood pressure was initiated 32 minutes after application of pressure and was maintained until release of pressure, 45 minutes after its application. Blood pressure fell rapidly after release of the pressure in the jacket and in 6 minutes had reached its initial level. Twilight alone produces no significant blood pressure changes.

Discussion. It seems apparent that a rise in blood pressure occurs in the human subject beginning 25 to 45 minutes after, and reaching its maximum 35 to 60 minutes after a pressure exceeding systolic blood pressure is applied to the forearm and hand. This rise apparently is not due to a somatic vasoconstriction. It has also been seen that this rise is not dependent upon any subjective reactions to the conditions of the experiment. On the other hand, we have failed to obtain any significant changes in blood pressure as a result of briefer periods of kidney compression in either anesthetized or decerebrate animals. Before attempting to reconcile these findings let us consider briefly the possible significance of the unquestionably positive findings in the human subject.

First, it is apparent that the mechanism producing this response is not mechanical forcing of the blood from the arms into the restricted circulatory bed. The long latent period eliminates this explanation. It also shows that this is not an ordinary pressor reflex, which is immediate in its response. Just what the mechanism is is not clear. We might conceive that owing to some metabolic disturbance in the ischemic tissues they elaborate a toxic pressor substance which slowly accumulates until it is present in concentration sufficient to produce physiological effects. This substance must then act locally on the peripheral nerves, since it is excluded from the circulation. Its action cannot be that of adrenalin for a vasoconstriction of the forearms alone could not produce such a marked rise in blood pressure; furthermore a vasoconstriction in the area of production of this hypothetical substance could not raise the blood pressure, since this area is already excluded from the circulation by the external pressure. We may present then, the conjecture that the hypothetical toxic substance acts upon the afferent fibers, producing an increasing pressor reflex as it accumulates. When the circulation is restored to the forearm this substance may be quickly oxidized, permitting the blood pressure to fall to normal. In the instances in which, while smoking, the blood pressure fell to a subnormal level and

collapse seemed imminent while the pressure was still being exerted upon the arm, it may be supposed that vasomotor fatigue supervened and perhaps was aggravated by the nicotine.

The region of vasoconstriction is probably splanchnic. The plethysmograph observation referred to above, in which it was seen that the volume of one forearm increased while the other was under compression and the blood pressure was rising, shows that the vasoconstriction does not include the opposite arm and presumably, therefore, none of the so-called somatic area, although it is recognized that the latter presumption must be made with considerable reservation. It is highly probable, then, that by far the most powerful vasomotor influence is exerted upon the splanchnic area and that the increase in volume of the opposite arm is purely passive.

In regard to our failure to get positive results in the animal experiments, it is apparent in the first place that the two sets of experiments are not analogous in that the time of compression in the animal experiments was not as long as the time required for the rise in blood pressure to make itself manifest in the human experiments. Longer periods were not used because it became evident that a compression of the kidney lasting 30 minutes would injure it so severely that even if a rise in pressure did occur we would not be justified in assuming that it was due to the same factor operating in our human experiments and also because we would have no return to normal conditions. We therefore felt that it was not practicable to carry out on an animal's kidney the same procedure carried out on a man's forearm.

We had conducted two experiments on rabbits, with negative results, when we found that Alwens (6) had preceded us in this method. Alwens found that on subjecting both kidneys of a cat to a pressure above systolic blood pressure there was an *immediate* rise in blood pressure of a few mm. (5 to 10) and an immediate fall in blood pressure on release of compression pressure. The latent period was imperceptible. He finds this occurring even after the splanchnic nerves have been cut, and that it disappears on ligation of the renal arteries. He does not ascribe any importance to this slight rise as an explanation of clinical hypertension, admitting that it is certainly due to a direct transmission of the pressure exerted upon the kidneys out through the renal arteries to the aortic stream. The reason for our failure to obtain a similar rise on kidney compression is due to the fact that we compressed only one kidney, the mechanical effect of this not being great enough to produce an appreciable change. In a case where Alwens compressed only one

kidney he obtained a rise in blood pressure of 4 mm. Hg. Such a rise cannot be regarded as of any significance; in fact, we obtained changes of this magnitude without attributing any significance to them. Alwens failed to obtain blood pressure changes on compression of the hind limb of dogs for brief periods. This he explained as being due to the indirectness of the transmission of the pressure to the aortic stream. It is obvious that the phenomenon with which we are dealing in our 40 minute periods of compression of the forearm is quite different from that dealt with by Alwens. As we have stated, we found that practical difficulties rendered it impossible to subject the kidney to a compression of the same duration as was practicable with the forearm. We may, then, reject both Alwen's and our own animal experiments as having no bearing on the phenomenon under consideration.

The idea that a rise in blood pressure might be a compensatory response to a local ischemia produced by a contraction of scar tissue, as in chronic interstitial nephritis, is, of course, an old one. So far as we can discover, however, Alwens was the first to attempt to reproduce this condition experimentally, and his attempt was unsuccessful. Our positive results were obtained by subjecting tissues to a much longer period of compression; and it is this difference in the method employed that undoubtedly accounts for the difference in results of previous observers and of ourselves. Alwens assumed that the response would be similar to that of intracranial tissue to compression, i.e., almost immediate, and his work was discontinued upon failure to get this immediate response. So far as we are aware ours is the first demonstration of a response by an increase in blood pressure to local ischemia produced by compression of extracranial tissue. We believe that there is a strong probability of a connection between these results and the mechanism of certain types of clinical hypertension. We feel that it is impossible to produce experimentally in the kidneys by compression conditions similar enough to those obtaining in kidney disease to subject our views to a fair test on kidney tissue, but we believe that we have succeeded in producing an analogous condition in the arm tissue, with strikingly positive findings.

SUMMARY

Compression of the forearm great enough to produce a local ischemia produces a rise in systolic and diastolic arterial pressures, the systolic pressure tending to rise above the compressing pressure.

The rise in pressure does not manifest itself until the compression has lasted 15 to 45 minutes, and then is usually maintained as long as the compression is maintained, at least up to 60 minutes. It appears earlier with smoking than without. Headache, sweating and a sensation of heat usually accompany the rise in pressure; in smoking nausea also is usually present, even in the case of habitual smokers.

Marked waves of blood pressure have been observed to initiate the rise. Whether or not this is a constant phenomenon is not certain.

The arterial pressure rapidly returns to its initial level on release of compression. In 2 cases the blood pressure fell to or below its initial level before decompression. This was presumably due to vasomotor fatigue and was accompanied by symptoms of collapse.

The rise in pressure is probably due to splanchnic vasoconstriction. It is accompanied by an increase in volume of the opposite arm, which is probably passive.

No significant change in blood pressure resulted on brief compression of the kidney in the anesthetized rabbit and dog, or in the decerebrate cat.

The suggestion is made that these findings may have some bearing on the etiology of clinical hypertension.

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THE SIGNIFICANCE OF HYDREMIA IN THE SECRETION OF URINE

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It is generally assumed by adherents of the modern theory of urinary secretion (1) that the diuresis following the drinking of large quantities of water is due to a dilution of the blood. The decrease of the osmotic pressure of the blood plasma incident to the hydremia, it is believed, favors filtration through the renal capsule.

There is, however, little direct experimental evidence to support this assumption. Engel and Scharl (2) studied the blood concentration and urine output after 1300 to 1400 cc. of fluid by mouth. Their patients showed diuresis but no diminution in the refractive index of the blood. In some of their experiments the blood became more concentrated during the height of the diuresis. MacCallum and Benson (3) produced profuse diuresis by drinking 2700 cc. of water. They also were unable to detect a diminution in the concentration of the blood with the hemocytometer. Haldane and Priestley (4) and Priestley (5) induced diuresis by drinking large quantities of water and saline. They found that the hemoglobin remained unaltered after water drinking but a slight reduction in the electrical conductivity of the serum occurred. After saline the hemoglobin was slightly diminished and the electrical conductivity of the serum increased. The diuresis after saline was less intense but more prolonged than after water drinking. They concluded that the diuresis following the drinking of fluids was not due to a dilution of the blood.

Ginsberg (6) studied the relation between hydremia and diuresis by comparing the amounts of urine secreted after water and saline drinking and after intravenous injections of the same substances. He found that the diuresis produced by giving the fluids intravenously was much less marked than that produced by giving smaller amounts by

mouth. He concluded that hydremia was not the important factor in causing water and saline diuresis. Cow (7) confirmed Ginsberg's findings and isolated a diuretic substance from the duodenal wall. He believed that when fluids were drunk this diuretic substance was washed into the circulation and produced the diuresis.

During the course of some studies on the fluid treatment of infants with diarrhea, data on the relation between blood concentration and urine output were obtained. These seem of sufficient importance to be reported here.

The method of study was as follows: Normal male infants were given 30 to 35 cc. of fluid per kilogram of body weight and the urine collected for four hours. Blood samples for refractometric determination were taken immediately before and at frequent intervals after the fluid was drunk. The water was sweetened with a little saccharine. Saline was given by gavage.

The serum concentration was determined with the Abbe refractometer. To prevent temperature variations, all the specimens from a single experiment were read simultaneously. Readings were made at temperature of 19 to 21° and distilled water was read each time. Blood was collected in Wright capsules. These were sealed and kept on ice.

The method of Robertson (8) was used to determine the refractive index of the non-protein substances of serum. The method consists of the precipitation of the serum proteins with weak acetic acid and heat. The refractive index of the acetic acid filtrate and of weak acetic acid are determined. From the difference between these two readings the refractive index of the non-proteins is calculated. We found that in the serum of well infants this ranged between 0.0016 and 0.0024. It was generally around 0.0020. We consequently used this latter figure in our calculations. 0.0020 was added to the reading for the refractive index of water, the sum was subtracted from the reading for whole serum. This gives the refractive index of the serum proteins. By dividing this figure by 0.00172, the value given by Reiss (9) for the refractive index of 1 per cent of protein, the percentage of protein is obtained. The method of calculating the serum protein percentage from the refractive index of serum may be represented as follows:

S = Refractive index of serum.
W = Refractive index of water read at the same temperature as the serum.
0.0020 = Refractive index of the non-protein substances of serum.
0.00172 = Refractive index of 1 per cent of serum protein (Reiss).

P = Refractive index of the serum proteins.

P = S − (W + 0.0020).

$\frac{P}{0.00172}$ = Percentage of serum protein.

Urine was collected by means of a curved glass tube, one end of which was drawn over the infant's penis and attached to the pubis and perineum with adhesive plaster. The other end was attached by means of glass and rubber tubing to a bottle which hung at the side of the bed. An electric buzzer announced the time of each voiding. The head of the bed was raised and the infant's legs gently restrained. This apparatus has been in use at this hospital for some time and has been found a great convenience in the complete collection of urine.

TABLE 1

Showing the results in water drinking experiments

EXPERIMENT NUMBER	SERUM PROTEIN PER CENT BEFORE WATER DRINKING	LOWEST POINT TO WHICH SERUM PROTEINS FELL AFTER WATER DRINKING	AMOUNT OF FLUID DRUNK	AMOUNT OF URINE VOIDED	PERCENTAGE OF INGESTED WATER EXCRETED IN FOUR HOURS
			cc.	cc.	
1	7.37	6.68	210	133	60
2	6.74	5.81	180	55	30
3	7.64	6.91	120	35	29
4	8.24	7.61	180	13	7
5	7.26	6.74	210	69	33
6	6.93	6.33	135	52	38
7	7.84	6.91	135	70	51
8	6.68	5.75	120	46	38
9	8.43	6.80	105	16	15
10	7.43	6.57	105	88	83
11	7.09	6.91	135	82	61
12	8.02	7.84	210	220	105

Results. The results of 12 experiments on 10 infants after water drinking are shown in table 1. (See also protocols at the end of the paper.) In 10 of the 12 experiments there was a distinct hydremia as indicated by a diminution in the refractive index of the blood serum. This started immediately after the water was drunk, reached its height in from thirty minutes to two hours and then gradually disappeared. The maximum dilution usually amounted to about 0.5 to 0.9 per cent of protein but in one instance the decrease in serum protein amounted to 1.63 per cent (exper. 9). In experiments 11 and 12 there was no significant change in the blood concentration.

In but 1 of the 12 cases was there excessive secretion of urine. In the others the amount of urine voided during the four-hour period following the water drinking varied from 7 to 83 per cent of the water drunk. Usually about 35 per cent was excreted. In experiment 9 where the serum protein dropped from 8.43 per cent to 6.80 only 15 per cent of the water drunk was excreted in the four-hour period. Baby E. T. (exper. 12), whose blood showed no significant diminution in concentration after the water, voided 105 per cent of the water drunk.

Seven experiments were conducted after saline gavage (table 2). In two instances no significant dilution of the blood occurred. The remaining five showed distinct hydremia. As after water drinking, the blood dilution started soon after the saline had been given and reached its height from thirty minutes to two hours afterwards. In

TABLE 2

Showing results in saline gavage experiments

EXPERIMENT NUMBER	SERUM PROTEIN PER CENT BEFORE WATER DRINKING	LOWEST POINT TO WHICH SERUM PROTEINS FELL AFTER WATER DRINKING	AMOUNT OF SALINE GIVEN	AMOUNT OF URINE VOIDED	PERCENTAGE OF INGESTED SALINE EXCRETED IN FOUR HOURS
			cc.	cc.	
1	5.81	5.58	200	0	0
2	7.26	5.99	200	24	12
3	7.31	7.03	180	18	10
4	6.39	6.57	180	17	9
5	7.20	6.54	200	0	0
6	7.55	5.93	100	14	14
7	7.67	6.51	200	31	16

three instances the hydremia was preceded by a brief period in which the blood became concentrated. The blood concentration in 4 experiments did not return to its pre-fluid level in four hours (table 2, exper. 2, 5, 6, 7). The hydremia after saline gavage was more marked than after water drinking, amounting, in 4 of the 7 experiments, to over 1 per cent of protein.

Following the saline there was a distinct oliguria. The largest amount of urine secreted in four hours was 16 per cent of that given. In two instances (exper. 2 and 5) no urine was voided during the four-hour period. These results correspond with those of Ohlman (10), who similarly found a retention of fluid after saline drinking in infants.

In our series of experiments, then, hydremia without diuresis was produced. In general, in those instances where the blood dilution was

greatest the urine output was smallest. After the saline gavage where the serum often fell more then 1 per cent the oliguria was marked. It seems clear that the diminution in plasma protein concentration and the consequent decrease in the osmotic resistance of the plasma to filtration through the renal capsule are not sufficient to cause diuresis in well infants.

SUMMARY AND CONCLUSIONS

1. The concentration of the blood and the secretion of urine were studied in a group of well infants after water and saline drinking.

2. After water drinking a moderate hydremia occurred without diuresis.

3. After saline drinking there was a marked blood dilution and oliguria.

4. In infants, hydremia alone does not necessarily cause diuresis.

A few illustrative protocols are appended.

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WATER DRINKING EXPERIMENTS

Experiment 4. Hydremia with oliguria after water drinking

R. A. Age 6 months, weight 5.7 kgm., 180 cc. water

TIME	SERUM PROTEIN	AMOUNT OF URINE
	per cent	*cc.*
9:55 a.m.	8.24	
10:01–10:20 a.m.	Water drinking	
10:20 a.m.	7.84	
10:32 a.m.	7.73	
11:01 a.m.	7.61	
11:54 a.m.	7.61	
12:00 m.		9
1:00 p.m.	8.18	
1:40 p.m.		4
Total urine		13

Experiment 9. Marked hydremia with oliguria after water drinking

H. S. Age 4 months, 4.6 kgm., 105 cc. of water

TIME	SERUM PROTEIN	AMOUNT OF URINE
	per cent	*cc.*
10:15 a.m.	8.43	
10:20–10:25 a.m.	Water drinking	
11:00 a.m.	7.61	
11:45 a.m.	6.96	
12:20 p.m.	6.80	
12:30 p.m.		16
1:45 p.m.	6.90	
Total urine		16

Experiment 12. Diuresis without hydremia after water drinking

E. T. Age 8 months, 6.4 kgm., 210 cc. of water

TIME	SERUM PROTEIN	AMOUNT OF URINE
	per cent	*cc.*
10:10 a.m.	8.02	
10:14–10:20 a.m.	Water drinking	
10:20 a.m.		19
10:25 a.m.	8.19	
10:50 a.m.	7.84	30
11:00 a.m.		40
11:20 a.m.	8.24	
11:50 a.m.		57
12:00 m.	8.02	
1:07 p.m.		26
1:15 p.m.	7.84	
2:50 p.m.	8.02	
2:15 p.m.		48
Total urine		220

SALINE GAVAGE EXPERIMENTS

Experiment 2. Marked hydremia and oliguria after saline gavage

Age 5 months, weight 4.7 kgm., 200 cc. of saline

TIME	SERUM PROTEIN	URINE
	per cent	*cc.*
10:05 a.m.	7.26	
10:11–10:13 a.m.	Saline gavage	
10:14 a.m.	7.61	
10:25 a.m.	5.99	
10:35 a.m.	6.51	
11:06 a.m.	6.68	
12:12 p.m.	6.57	
1:03 p.m.	6.74	
2:07 p.m.	6.74	
2:15 p.m.		24
Total urine		24

Experiment 6. Marked hydremia and oliguria after saline. Palmer method used for the determination of hemoglobin

T. P. Age 3 months, 3.6 kgm., 100 cc. of saline

TIME	HEMOGLOBIN	SERUM PROTEIN	URINE
	gm. per 100 cc.	*per cent*	*cc.*
10:15 a.m.	15.0 gm.	7.55	
10:30 a.m.	Saline gavage		
11:00 a.m.	13.5 gm.	5.93	
11:30 a.m.	14.6 gm.	6.68	
12:15 p.m.	15.0 gm.	6.91	14
1:45 p.m.	14.1 gm.	6.57	
Total urine			14

A STUDY OF THE REGENERATION OF THE AUTONOMIC FIBERS IN THE VAGUS NERVE OF THE SHEEP

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Although various incomplete summaries of the work done on the regeneration of the cardiac fibers in the vagus nerve have appeared at different times, it may not be amiss to give the actual conclusions reached together with the definite data from which these were drawn.

As early as 1878 Gluck (1), working with rabbits, was under the impression that he had established the certainty of a vicarious function in the cardiac fibers of the vagus in the case of one animal, within 10 days after cutting. He also found that in other instances manipulation of a severed and sutured vagus gave active swallowing movements some time later, "partiellen Restitution der Function." No definite time for recovery of function is stated in these cases. However it clearly antedates the work of Vanlair (2).

The latter found that when the vagus in a dog was cut and the ends sutured together, he obtained recovery of function in the recurrent laryngeal branch in 11 months. He was able to demonstrate no return of function in autonomic fibers.

Tuckett (3) first either cut or crushed the left vagus nerve in 4 rabbits and found that he got no regeneration of autonomic fibers, such that electrical stimulation of these nerves would cause a change in heart rate, in from 89 to 231 days. Later however (4) he reported that in the case of 3 rabbits in which he had either cut or crushed the left vagus, he was able to produce slowing of the heart by stimulation of the injured nerve in 2 of them after from 3 years, 8 days to 3 years, 38 days. This is the first authentic record of regeneration of the autonomic cardiac fibers which has come to the writer's attention.

In a dog, Stewart (5) found evidence of cardiac response to stimulation of the right vagus nerve, which had been cut 300 days before.

This work was repeated by Rogers (6) on 4 cats and 3 dogs. In the cats he found no regeneration in from 2 to 6 months and in 2 of the

dogs none in from 4 to 16 months. In the third dog however the right vagus had been cut and in 20 months the heart was definitely slowed by stimulation of this injured nerve.

Langley (7) cut the right vagus in a kitten and found no functional regeneration after 12 months.

In Schafer's laboratory the most extensive study of the subject has been carried on by several investigators. Tsukaguchi (8) severed the right vagus in 2 dogs and failed to get cardiac response upon stimulation 173 to 175 days later. Schafer and Feiss (9) cut the left vagus in 2 cats and stimulated the severed nerve 12 months, 9 days to 13 months later; cut the right vagus in 3 cats and examined 10 to 11 months later; severed the right vagus in 1 rabbit and investigated 74 days afterward; cut the right vagus in 1 dog and examined 11 months later. In no case was regeneration of cardiac fibers indicated.

Schafer (10) published the results of his own work in which the left vagus of a dog was cut and examined 1 year, 10½ months later. The right vagus was cut in 5 cats and investigated from 1 year, 5 months to 2 years, 50 days later. No evidence of functional recovery of the nerve was found in either the cats or the dog.

Up to date, all of the work upon regeneration of the autonomic fibers in the vagus has shown that the cardiac fibers in the right vagus of the dog may in some instances again become functional in from 300 days to 20 months, and that similar results may follow severing the left vagus of the rabbit in from 3 years, 8 days to 3 years, 38 days.

Experimental. In December, 1920, 5 sheep, 4 ewes and 1 ram were procured for observation. The normal heart and respiration rates were obtained by keeping the animals quietly standing in the corner of a room for 10 or 15 minutes. Then the heart rate was determined by auscultation. When all of the results were satisfactory, a unilateral vagotomy was performed upon each sheep.

In two ewes the left vago-sympathetic trunk was cut and in the remaining two ewes and the ram the right vago-sympathetic was severed. All nerves were cut at the level of about the middle of the thyroid gland.

In the sheep the cervical sympathetic is usually so closely bound to the vagus by connective tissue that, to avoid possible injury to both nerves, both were cut across instead of the vagus alone. After cutting, the ends of the trunks were approximated and held by a fine silk suture.

Before tying, however, the peripheral ends of the cut nerves were stimulated by induced current and the heart rate observed by placing the bell of a stethoscope over the apex region.

During the operation the sheep was placed upon its back and held. The field of incision was infiltrated with a 1 per cent solution of apothesine. It was found that about 7 cc. of this solution gave satisfactory local anesthesia.[1]

To avoid the death of the sheep by asphyxiation in case the cutting of the one inferior laryngeal nerve caused occlusion of the larynx, due to collapse of a thyreo-arytenoid ligament, trachea tubes were inserted in two of the ewes before the incision was sutured. These tubes were cleaned daily.

After 5 or 6 days, observations were again made upon the heart and respiration rate, with each animal quiet, as before the operation.

Three ewes and the ram died from 1 to 8 months after operation.

On December 8, 1921, 363 days after the right vago-sympathetic trunk had been cut and sutured, the remaining ewe was brought up for observation.

The sheep was anesthetised with ether, a tracheal cannula placed in position and ether alone was continued throughout the experiment.

A glass cannula was placed in the right common carotid artery and connected with a mercury manometer. Another cannula was placed in the right femoral artery and attached to a Hürthle manometer. A pneumograph was arranged to record abdominal respiration. The manometers and the recording tambour of the pneumograph were arranged to record on the same vertical line with a signal magnet and time recorder, on a long paper kymograph. It will be noted in the tracing that the signal magnet was accidentally displaced slightly to the right of the line of the other recording instruments.

Cohn (11) lead electrodes were placed on the right fore leg and left hind leg so that simultaneous electrocardiograms could be taken.

The right vago-sympathetic trunk was cut and the peripheral end repeatedly stimulated with varying strengths of tetanizing current. Then the left vagus trunk (exclusive of the sympathetic) was tied, cut and stimulated.

Discussion. Of the five sheep operated upon, four died. Three were old sheep and were used for this experiment in the hope that they might live to its completion. Their deaths were probably not due to the operation. Two were young healthy ewes and of these one died of pneumonia following lambing. The remaining ewe was the one investigated.

[1] Dr. Martin B. Tinker kindly advised on this method of anesthetising rather than a general anesthetic, in order to avoid pneumonia following the operation.

In a comparison of the respiration and heart rate while the sheep were standing quietly, it was found that in the four cases in which records had been kept the heart rate was about the same before and a week following the operation. The respiratory rate was in every case from 4 to 10 per minute less, the week following vagotomy.

In the case of the previously severed vago-sympathetic nerve trunk in the ewe examined, it was found that ligature and cutting followed by stimulation of the peripheral end with weak and strong induced current produced no effect upon the heart rate. This would lead to the conclusion either that there is no regeneration of the efferent autonomic fibers of the vagus in the sheep or that regeneration is not complete in 12 months.

It will be noted that in figure 1 the first application of a long-continued stimulus to the peripheral end of this right nerve resulted in a decrease in pulse pressure, indicated by the Hürthle manometer. No change of course was shown by the mercury manometer, since this does not register pulse pressure.

It might be possible to explain the results of Johansson and Tigerstedt (12) in which they found that stimulation of the left vagus of a cat resulted in a decreased force of contraction of the heart, by assuming the presence of more than one kind of fiber in the vagus. If this should be true in the sheep, it is possible that complete regeneration of this type of fiber had occurred and caused the decrease in pulse pressure. This explanation is rendered rather improbable by the work of Erlanger (13).

It is more probable that the escape of this strong stimulus to surrounding tissue caused a momentary increase in vasomotor tonus with a resulting decrease in pulse pressure.

Light stimulation of the peripheral end of the right (normal) severed vagus shows slowing of the heart. It is the typical picture of cardiac inhibition with vagus escape (see fig. 2).

The fact that the pneumograph was applied around the abdomen would render questionable the assignment of explanations for the peculiarities of the respiratory curve at the last stimulation of the left vagus. It would be so easy to obtain distorted records through normal functional movements of the abdominal viscera.

Upon stimulation of the peripheral end of the right (normal) vagus, there is a drop in the respiratory curve. This drop may be due to the sudden motor stimulation of the stomach, resulting in violent contraction with consequent traction upon the diaphragm at the cardia. This would increase the size of the abdomen by visceral compression, giving this type of curve.

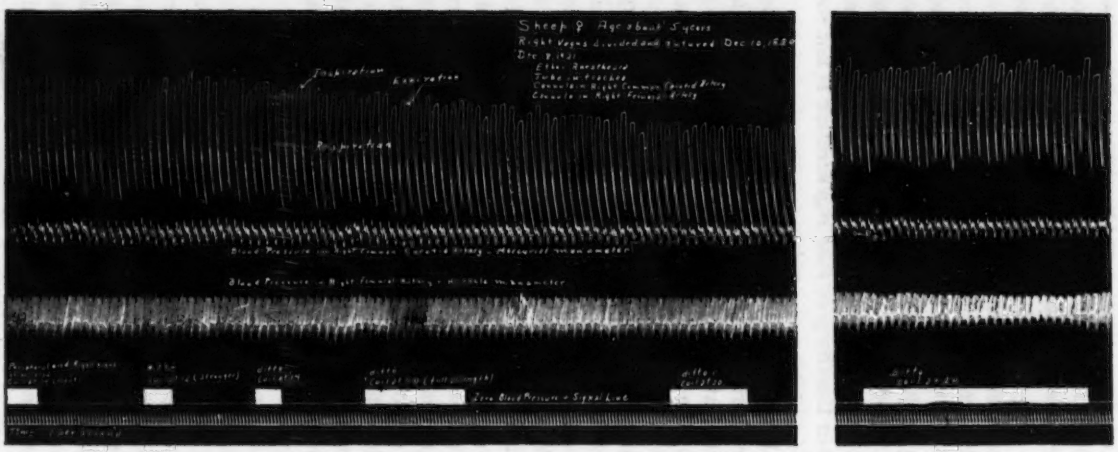

Fig. 1. Blood pressure (Hürthle and mercury manometers) and respiratory tracings during stimulation of the peripheral end of the right (injured) vagus nerve.

It will be noted in figure 2 that there was a distinct slowing and deepening of respiration after tying the first ligature on the right (normal) vagus nerve. Although the rate increased slightly within a few minutes, it did not return to normal during the course of the experiment—about 3 hours, nor did the rate increase. This would indicate that in this sheep cutting of the one normal vagus and functional inaction of the other showed the classical respiration picture of double vagot-

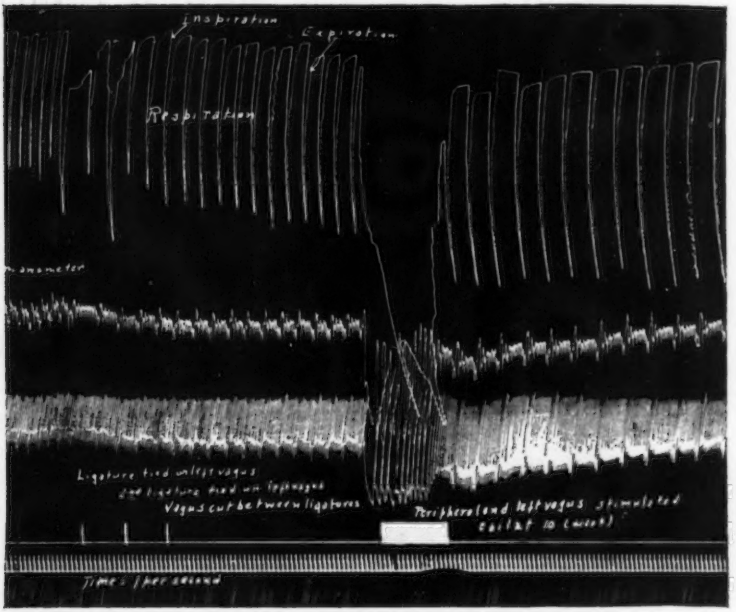

Fig. 2. Blood pressure and respiratory tracings during stimulation of the peripheral end of the left (normal) vagus nerve.

omy, despite the presence of the tracheal cannula. This must be contrasted with the majority of results as reported by Schafer (10) for animals of some other species.

CONCLUSIONS

There is probably no functional regeneration of the autonomic fibers in the right vagus nerve of the sheep within a 12-month period.

With a tracheal cannula inserted, the cutting of the second vagus nerve gave the "classical" picture of respiration rate and amplitude following double vagotomy.

I wish to thank Dr. Sutherland Simpson for help in carrying out these experiments, and Mr. B. R. Macmillan, department mechanician, for assistance with the apparatus. I also wish to acknowledge my indebtedness to Mr. S. H. Bassett for assistance at the operations.

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THE SECRETORY ACTION OF THE PANCREAS IN RELATION TO THE THYROID GLAND

I. The Effect of Thyroid Feeding in Rats upon the Secretory Action of the Pancreas

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It has been noticed by a number of investigators that thyroid-feeding produces certain effects upon the digestive tract of animals. Carlson (1) and Farrant (2) observed in thyroid-fed animals diarrhea and a hemorrhagic condition of the intestines in spite of the increase in appetite. Schafer (9) found that thyroid-feeding causes in growing white rats an increase of food-consumption and an acceleration of bodily growth. Hewitt (4) noticed that the food-consumption of white rats diminished, when comparatively large quantities of thyroid were given, while on the contrary comparatively small quantities caused the animals to consume more food and gain more weight in body than normal. Marbé (7) observed that thyroid administered to dogs acted as a stimulant to the secretory action of the intestinal glands, the quantity of the intestinal juice secreted increasing markedly. Nürenberg (8) fed iodo-thyro-globulin to dogs, and found the secretion of the intestinal and pancreatic juices accelerated and augmented during digestion and for a while after, whereas such changes were not shown by the control animals, which were fed with inorganic iodine compound. A hemorrhagic condition of the intestinal mucous membrane appeared in these thyroid-fed animals at the same time. Kojima (5) found in the pancreas of thyroid-fed white rats morphogenetic changes indicative of rapid multiplication of the gland-cells and at the same time considerable diminution of the zymogen-granules. Determining the amylase-content of the pancreas in thyroid-fed white rats I (3) attempted to investigate the changes in the secretory action of the pancreas resulting from thyroid-feeding. And I observed that thyroid-feeding resulted in a marked decrease of amylase-content of the pancreas.

In view of Hewitt's observations of the changes in appetite resulting from feeding different amounts of thyroid, however, it was thought well to investigate further how widely different effects upon the secretory action of the pancreas would be caused by different doses of thyroid administered. Hence in the present investigation white rats of nearly similar size were divided into a number of groups to be fed with different quantities of thyroid, and the changes of the secretory action of the pancreas resulting from thyroid-feeding in the animals of different groups were compared with one another.

Method. The rats employed for experiments were all fully grown ones which fed on mixed bread and milk in equal amounts and greens. They were kept in wire-net cages, being separated from one another. Thyroid administered was desiccated thyroid powder containing 0.488 per cent of iodine, I myself made from fresh ox thyroid glands, and various amounts of it—from 0.5 gram to 0.03 gram per day to each animal—were added to the bread-and-milk mixture. To the control animals were given similar amounts of desiccated beef. The effects of thyroid-feeding upon the general conditions of the animals were observed carefully, viz.: changes of body-weight, general behavior, appetite, excreta, etc. After thyroid was fed for a certain length of time, the animals were killed by chloroform and bled. Immediately after death the body-length was measured, i.e., the distance from the tip of the nose to the center of the anus.

The pancreas was removed in one piece, freed from the adjacent tissues, weighed, and then rubbed in a mortar with fine sand and saline solution. The mixture was rinsed into a flask with more saline solution, using in all a hundred parts by volume (cc.) of saline solution to one part by weight (gram) of gland substance. The flask was plunged into a water-thermostat at 38°C. After an hour the mixture was filtered through paper, resulting in a clear or slightly opaque solution. The extract of pancreas thus obtained was used for determining its amylase-content.

The small intestine in its entire length was removed, ligatures being at both ends, and was slit up with a pair of scissors. Its contents *in toto* were removed, weighed, and mixed in a mortar with the saline solution in the proportion of one part by weight (gram) of juice to ten parts by volume (cc.) of saline solution. The mortar containing the mixture was set in an ice-box for an hour, and then the homogeneous mixture was filtered through paper. The solution of intestinal juice thus obtained was used for determining its amylase-content. The

saline solution used was the physiological saline solution, to which was added one-fifteenth part of the solution consisting of one part of a normal solution of acetic acid and thirty-two parts of a normal solution of sodium acetate.

The quantitative determination of amylase was made according to Wohlgemuth's method. The starch-solution used was of 1 per cent. It was made of Merck's soluble starch instead of Kahlbaum's, inasmuch as the former had proved to give results similar to those of the latter. The duration of digestion was limited to 30 minutes, so that the spontaneous deterioration of amylase might be avoided as much as possible. As for formulating the results obtained by this test, the amylolytic activity would be expressed by the amount (cc.) of 1 per cent starch-solution digested by 1 cc. of ferment-solution during 30 minutes at a temperature of 38°C. In order to indicate the quantity of amylase in the following accounts of my experiments, the unit was fixed at such an amount of amylase as would digest 1 cc. of 1 per cent starch solution completely during 30 minutes at a temperature of 38°C.

Results. The amylase-content of the pancreas in sixteen normal, fully grown, male, albino rats quoted as the controls was determined quantitatively to be as following. The amount of amylase per 1 gram of gland substance varied from 21,700 to 53,400 units, and averaged 30,900 units. The amount of pancreas-amylase per 100 mm. of body-length ran from 8,700 to 20,000 units, and that per 100 grams of body-weight, from 7,090 to 15,900 units, the average being 13,300 and 11,400 units respectively. The amylase-content of the pancreas in four control female rats (non-pregnant) varied from 20,800 to 50,000 units per 1 gram of gland-substance, and averaged 31,000 units; the amount of pancreas-amylase per 100 mm. of body-length, from 10,740 to 18,500 units, the average being 13,300 units, and that per 100 grams of body-weight, from 9,790 to 23,750 units, the average being 14,900 units.

As the result of feeding rats comparatively large doses of thyroid—from 0.2 to 0.5 gram of desiccated thyroid daily to each, viz., from 0.08 to 0.21 gram of desiccated thyroid per 100 grams of body-weight—the amylase-content of the pancreas both in the males and in the females was notably diminished. In the majority of these cases the amylase-content of the pancreas was found to have decreased far beyond the lowest limit of the normal variation as shown by the control animals. The percentage reduction in the amount of amylase per unit-weight of gland substance, viz., the concentration or density of amylase in the gland substance, averaged from 55 to 80 per cent in

TABLE 1

The average amylase-content and the average weight of the pancreas in the thyroid-fed rats grouped according to dosage of thyroid

LOT, SEX	NUMBER OF RATS IN EACH LOT	THYROID-FEEDING		AVERAGE FINAL BODY LENGTH	AVERAGE FINAL BODY WEIGHT LENGTH RATIO	AVERAGE LOSS OF BODY WEIGHT PER 10 DAYS	PANCREAS		
		Dose per day	Average duration in day				Average weight per 100 mm. body length	Average amylase content	
								Per 1 gram gland substance	Per 100 mm. body length
		grams		*mm.*			*grams*		
I M	6	0.5	9	200	0.96		0.388	13,300	5,600
						(−19)	(−11)	(−57)	(−58)
II M	2	0.3	11	187	0.82		0.486	6,300	3,100
						(−16)	(+11)	(−80)	(−77)
III M	2	0.2	5	198	1.10		0.410	13,900	5,800
						(−43)	(−6)	(−55)	(−57)
IV M	2	0.1	15	192	0.82		0.488	33,300	16,100
						(−19)	(+11)	(+8)	(+21)
V M	4	0.03	112	198	1.00		0.457	30,900	14,400
						(−1)	(+4)	(±0)	(+8)
VII M	16	Controls		206	1.14		0.438	30,900	13,300
VIII M	6	0.3	11	190	0.88		0.620	12,600	8,000
						(−20)	(+35)	(−59)	(−39)
IX F	5	0.03	82	188	0.96		0.546	32,400	18,100
						(−2)	(+19)	(+4)	(+37)
X F	4	Controls		191	0.95		0.457	31,000	13,200

* The unit of quantity of amylase is fixed at such an amount of amylase as would digest 1 cc. of 1 per cent starch solution completely during 30 minutes at a temperature of 38°C.

The figures in brackets indicate the percentage differences from the control values.

each lot. In the extreme case, the decrease amounted to about 95 per cent of the average amylase-content as shown by the controls, and to 94 per cent of the low limit-value of the controls. The amount of pancreas-amylase per 100 mm. of body-length or per 100 grams of body-weight was similarly reduced, most of the figures obtained for those

being far lower than the lowest figure given by their controls. Since thyroid-feeding interfered considerably with the normal gain of body-weight in these cases, the comparison of the amount of amylase per unit of body-length probably gives a more exact figure of the changes which took place than a comparison in terms of body-weight does. The percentage decrease in the average amount of pancreas-amylase per 100 mm. of body-length obtained for each lot varied from 39 to 77 per cent of the control value. In the extreme case the reduction was 95 per cent of the average control value, and 92 per cent of the lowest control value.

On the other hand, the majority of the animals that had received greatly reduced amounts of thyroid—each 0.1 gram of desiccated thyroid daily (0.05 gram per 100 grams of body-weight) or each 0.03 gram of desiccated thyroid on alternate days (from 0.010 to 0.033 gram and from 0.013 to 0.020 gram per 100 grams of body-weight in the males and in the females respectively)—showed a tendency to have an abundance of amylase stored in the pancreas, although none of them showed greater values than the highest limit of the normal fluctuations as shown by the controls. The average amounts of pancreas-amylase per 100 mm. of body-length for these lots were larger than the averages of the corresponding controls by from 8 to 37 per cent. Since the amounts of amylase per unit-weight of gland substance, viz., amylase-concentration on the gland substance, in these cases, were not at all or only slightly increased—on the average from 4 to 8 per cent—these increases of amylase in the pancreas of these animals must be attributed in no small degree to the enlargement of the gland (hypertrophy), which had been caused by thyroid-feeding. The weight of the glands per 100 mm. of body-length in these thyroid-fed rats averaged from 4 to 19 per cent higher than those of their controls.

For the purpose of making the nature of the changes in the secretory action of the pancreas more obvious, I further made use of the results obtained in determining the amylase-content of the intestinal juice. As is generally known, the amylase contained in the intestinal juice, or at least the largest part of it, originates in the pancreas-secretion. In all mammals the pure intestinal juice obtained from isolated loops of the small intestine contains invertase, maltase and occasionally lactase, but no amylase at all. Throughout the entire alimentary canal amylase is provided by no digestive fluid except saliva and the pancreatic juice. And as stated by Langley (6), the salivary amylase is destroyed eventually by the hydrolic acid contained in the gastric

juice, 0.003 per cent hydrolic acid—approximately pH = 3—being sufficient to destroy the salivary amylase. Hence it may be claimed that an increase or decrease in the amount of intestinal amylase indicates an increase or decrease in the amount of amylase given by the pancreas, provided H-ion concentration of the gastric juice be higher than pH = 3, strong enough to destroy the salivary amylase before it enters into the duodenum.

TABLE 2

The average amylase-content of the pancreas and that of the intestinal juice in the thyroid-fed rats grouped according to dosage of thyroid

LOT, SEX	NUMBER OF RATS	THYROID FEEDING		AVERAGE FINAL BODY LENGTH	AVERAGE LOSS OF BODY WEIGHT	PANCREAS			INTESTINAL JUICE		STOMACH CONTENT	
		Dose per day	Average duration in day			Average weight in grams per 100 mm. body length	Average amylase content		Average amount in grams per 100 mm. body length.	Average amylase content per 100 mm. body length	Weight	Acidity
							Per 1 gram gland substance	Per 100 mm. body length				
		grams		*mm.*							*gms.*	*pH*
I M	2	0.3	11	183	(−18)	0.486 (+8)	6,300 (−80)	3,100 (−76)	0.368 (+26)	118 (−53)	2.5	3.5
II F	2	0.3	17	186	(−19)	0.767 (+70)	16,800 (−45)	12,100 (−8)	0.450 (+55)	580 (+129)	0.5	1.5
III M	2	0.2	5	198	(−17)	0.416 (−7)	13,500 (−56)	5,600 (−68)	0.530 (+82)	338 (+46)	1.6	2.0
IV M	2	0.1	14	192	(−27)	0.489 (+9)	33,300 (+8)	16,000 (+21)	0.748 (+156)	1,090 (+331)	2.3	3.0
V MF	6	Controls		207		0.450	30,800	13,200	0.291	253	4.0	2.5

See the foot-note of table 1.

In the normal albino rats, when the stomach was filled with a fair amount of food and the small intestine with much turbid and thick chyme, i.e., at the period of intestinal digestion, the amount of amylase contained in the chyme present throughout the entire length of the small intestine varied from 144 to 433 units, and averaged 253 units per 100 mm. of body-length.

Among the thyroid-fed rats, observed under conditions similar to those of the controls, two males receiving 0.3 gram of desiccated thyroid daily for 8 and 13 days respectively, showed fair reductions in the amounts of intestinal amylase, one of which was obviously lower

than the minimum limit of normal variations as shown by the controls. The amounts of amylase contained in the pancreases of these two animals were found to be below the minimum limit of the normal fluctuations, both per unit-weight of gland and per 100 mm. of body-length. It is evident that the secretory action of the pancreas was severely impaired in the above cases.

On the other hand two male rats receiving 0.1 gram of desiccated thyroid daily for 10 and 17 days respectively showed tremendous increases in the amylase-content of the intestinal juice. The amounts of the intestinal amylase per 100 mm. of body-length were notably larger than the maximum amount shown by the controls, and averaged over 300 per cent higher than the average of the controls. The amylase-content of the pancreas in these animals was found within the limits of the normal variations as shown by the controls, although the average from these was a little higher than the control value. Such changes of the amylase-content of the pancreas by themselves indicate merely that there might be tendency toward an increase in the secretory action of the pancreas, but the increase of the pancreas-amylase is too small to prove that the secretory action of the gland was increased enormously as the result of thyroid-feeding. Judging from the results of determining the amylase-content of the intestinal juice, however, it is evident that there was a decided hyperfunction of the pancreas in the above cases.

Another interesting result was obtained in two male rats receiving 0.2 gram of desiccated thyroid daily for 3 and 6 days respectively, and two females receiving 0.3 gram of desiccated thyroid daily for 15 days and 19 days respectively. In the former two animals there were fair decreases in the amylase-content of the pancreas—below the limit of normal variations—but the amylase-content of the intestinal juice was by no means decreased, but was found to be within the limits of normal variations. The evidence that the intestinal juice contained a normal amount of amylase argues that the pancreas had been giving out into the intestine as much amylase as normal, although in the pancreas itself, when examined, was found very little amylase stored up. The most reasonable interpretation of the above changes would be that the gland was temporarily exhausted in its secretory action, after it had been functioning vigorously at the period of intestinal digestion. Now in the animals of the latter group the amylase-content of the intestinal juice was found to be rather markedly increased, even by a hundred per cent of the control value in spite of a considerable reduction in the

amylase-content of the pancreas. These changes suggest that the pancreas was exhausted in its function of producing amylase, after it had been producing and sending out larger amounts of amylase into the intestine than normal. The pancreas of these animals seems capable of meeting the demand on the part of the digestive tract for digestive ferments with an increased secretion, but it is soon exhausted, i.e., it is in a condition of increased secretory activity associated with increased exhaustibility.

From the above evidences we may infer that feeding animals certain small amounts of thyroid induces a profitable increase in secretory action of the pancreas and causes the gland to produce and send out greater amounts of digestive ferments than normal, but, if the dose of thyroid is increased beyond a certain limit, the gland is impaired, so that it cannot meet successfully the demand of the digestive tract for the ferments. And we may further assume that feeding certain intermediate amounts of thyroid changes the secretory action of the pancreas, so that the demand of the digestive tract for increased quantities of ferments may be met by the gland for a while successfully, but the pancreas itself will be easily exhausted in the process.

SUMMARY

Feeding animals comparatively large quantities of thyroid interferes notably with the secretory action of the pancreas, but on the contrary feeding comparatively small quantities causes a fair increase in that function.

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THE SECRETORY ACTION OF THE PANCREAS IN RELATION TO THE THYROID GLAND

II. The Effect of Thyroidectomy in Rats upon the Secretory Action of the Pancreas

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In my previous experimental researches into the effect of thyroid-feeding upon the secretory action of the pancreas, I (3) ascertained that feeding comparatively small amounts of thyroid causes an increase in the secretory action of the pancreas of albino rats, whereas comparatively large amounts of thyroid induce rather a decrease in the same. Now the question arises: Does the active principle of thyroid gland in the normal condition of the animal-organism also act as a hormone or an excitatory autacoid (6), increasing the rate of the action of the pancreas so as to maintain its normal secretory function? Do such amounts of thyroid-autacoid as are passed by the normal gland into the general blood-circulation exert an excitatory effect upon the secretory action of the pancreas? In solution of this problem, we need additional evidence to support the above data derived from the experiments in thyroid-feeding, inasmuch as many poisonous agents act as a stimulus to various physiological functions of the animal-organism when administered in very small doses, but are injurious when given in larger amounts. In order to establish the point in question, we must resort first to another method usually employed by investigators for the purpose of determining the function of the endocrine organs, viz., observation of the changes in the secretory action of the pancreas resulting from the surgical removal of the thyroid gland. If the active principle of the thyroid gland promotes the secretory action of the pancreas even in the normal condition of the animal, it may be expected that the absence of the thyroid gland would lead to an impairment of that action. The following experiments were made to ascertain the effect of thyroidectomy upon the secretory action of the pancreas.

Albino rats were employed for the experiments. The dietetic and environmental conditions of the animals during the experiments and the methods of determining the secretory action of the pancreas were altogether identical with those of my previous experiments. The thyroid glands were removed without anesthesia after keeping the animals for at least 2 weeks under the same dietetic conditions as those following the operation. When the parathyroids were found lying on the surface of the thyroid glands, these were removed and grafted deep in the operative wounds. The wounds usually healed without suppuration within 2 days. Thyroidectomized animals were killed at various periods after the operation, and the absence of the thyroid glands was verified by naked eye. When any formations simulating thyroid glands were discovered, these were examined microscopically.

The first series of experiments was performed on albino rats of four litters. Some of the operated ones showed symptoms of *tetania parathyreopriva* which usually appeared 2 or 3 days after the operation, stiffness and quivering of the limbs, and, occasionally, rapid, gasping respirations. Such animals died eventually, usually within 5 days. Only one of these lived as long as 18 days, when it was killed. A large number of the animals was lost after the removal of their thyroid glands without exhibiting tetany, so that only seven were available for measuring the secretory action of the pancreas. These animals were killed 15 and 17 days respectively after the operation. The thyroidectomized adult animals appeared to be entirely normal in the secretory action of the pancreas as well as in their general condition, no difference from their controls being evident. Among young adult animals the thyroidectomized one lost not a little of its body-weight, but its pancreas was apparently normal in its secretory action. In one rat which had exhibited the symptoms of chronic tetany, the pancreas and the intestinal juice contained fair amounts of amylase.

Judging from the results of the above experiments made on animals killed as late as 15 or 17 days after the removal of their thyroids, we are forced to conclude that the lack of thyroid secretion does not induce any changes in the secretory function of the pancreas. Cramer and M'Call (2) in their investigations of the effect of thyroidectomy in albino rats on the gaseous metabolism, ascertained that the metabolism after removal of the thyroid glands passes through two stages: At first, there is a reduction of the metabolism which is still reduced in the second week after the operation; and, later, an increased metabolism, produced probably by way of compensation. They regarded it as

reasonable to restrict the term "experimental hypo-thyroidism" to the early period in which the metabolism is reduced, as is usually seen in the pathological hypo-thyroidism of human subjects. If such a compensatory arrangement can be brought about so soon after the removal of the thyroid glands in albino rats, as demonstrated by the above investigators, then the results of my first series of experiments, as described above, are of little value in determining the changes in the secretory action of the pancreas due to the absence of thyroid secretion because, in order to measure the secretory action of the pancreas, the animals in the above experiments were killed more than 15 days after the removal of their thyroid glands, by which time the compensatory adjustment for the loss of the thyroid glands would already have been made.

Hence in the second series of experiments the animals were killed at earlier periods as well as at later periods after thyroidectomy, one of these being killed as early as 2 days after the operation, when the wounds had just healed. The animals employed were all fully grown male albino rats, taken from closely related litters of the same strain. After the operation comparatively few animals—about 20 per cent of the operated ones—suffered so severely from the operation itself as to be incapable of taking food properly on a subsequent day. The majority—55 per cent—lost their appetite rather later on the third day after the operation, when the wounds had completely healed. Nearly at the same period the animals became slightly inactive in general behavior. In none of these thyroidectomized animals, killed within 13 days after the operation, were thyroid glands found to be regenerated. But the animals, if killed more than 15 days after the thyroidectomy, exhibited in the neighborhood of the larynx small round or oval bodies that were about $\frac{1}{4}$ to $\frac{1}{2}$ of the normal thyroid gland in size and consisted of tissue quite similar to that of the normal thyroid glands. It was not certain whether the appearance of these formations was due to the regeneration of the gland tissue from the remnants of the removed glands, or hyperplasia of the accessory glands.

As to the secretory action of the pancreas, only the results obtained in the second series of the experiments were shown in the table. In an animal killed 2 days after the operation, when the wound had just healed, the amylase-content of the pancreas as well as of the intestinal juice was found to be quite large. In two animals killed 3 days after thyroidectomy the secretory action of the pancreas was found to be considerably reduced, one especially of these showing the amylase-

content of both the pancreas and the intestinal juice to be clearly less than the least shown by the controls. An animal killed at the end of the first week exhibited similarly a fair reduction. Comparing the

TABLE 1

Average amylase-content of the pancreas and that of the intestinal juice in thyroidectomized rats and their controls

	WEEK, IN WHICH RATS WERE KILLED, AFTER OPERATION	NUMBER OF RATS	AVERAGE FINAL BODY LENGTH	AVERAGE FINAL BODY WEIGHT LENGTH RATIO	PANCREAS			INTESTINAL JUICE		STOMACH CONTENT	
					Average weight per 100 mm. body length	Average amylase content		Average amount in grams per 100 mm. body length	Average amylase content per 100 mm. body length	Weight	Acidity
						Per gram gland substance	Per 100 mm. body length				
			mm.		*grams*					*grams*	*pH*
Thyroidectomized ...	First	4	185	0.785	0.275	33,400	9,400	0.713	545	0.5	2.5
	Second					(−43)	(−49)		(−37)		
	Second	4	182	0.710	0.339	52,300	17,600	1.000	660	1.4	2.8
						(−9)	(−2)		(−31)		
	Third	3	183	0.818	0.343	58,100	20,000	1.078	1,050	3.2	2.8
						(+17)	(+13)		(+11)		
Controls	First	2	189	0.810	0.319	58,600	18,400	1.165	865	1.0	1.0
	Second	4	180	0.728	0.307	57,800	18,000	0.785	964	0.6	2.8
	Third	3	191	0.860	0.355	49,700	17,700	1.140	945	1.7	2.8
Thyroidectomized...	First	8	183	0.748		42,000	13,500		603		
	Second					(−26)	(−26)		(−35)		
Controls	First	6	183	0.756		58,100	18,150		932		
	Second										

The unit of quantity of amylase is fixed at such an amount of amylase as would digest 1 cc. of 1 per cent starch solution completely during 30 minutes at the temperature of 38°C.

The figures in brackets indicate the percentage differences from the control values.

average amylase-content of the pancreas and the intestinal juice in all the animals killed within a week after thyroidectomy, with that in their proper controls, the reduction was estimated as of 49 and 37 per cent respectively.

In four animals killed in the second week after thyroidectomy, the amylase-content of the pancreas was found to be within the limits of normal fluctuations as shown by their controls, and the average figure differed but little from that of their controls. We may assume, however, that there was a fair reduction in the secretory action of the pancreas, for it was found that the amylase-content of the intestinal juice tended to decrease in the thyroidectomized animals, and the average was less by 31 per cent than that of the controls.

In three animals killed the third week after thyroidectmy, the amylase-content of the pancreas and the intestinal juice was found to be somewhat larger than that of their proper controls. As these animals all, however, exhibited regenerated thyroid glands in the place of the removed ones, it need hardly be pointed out that the observation of the secretory action of the pancreas in such animals can give us little information concerning the changes due to the absence of the thyroid-secretion.

The changes resulting from the absence of the thyroid-secretion should be expected only at an early period within 2 weeks after the removal of the thyroid glands, when no compensatory adjustment has yet been made. The average amylase-content of the pancreas, per unit-weight of the gland as well as per 100 mm. of body-length, in all the thyroidectomized animals, killed within 2 weeks after the operation, was less than that in their controls by about 26 per cent. The average amount of intestinal amylase per 100 mm. of body-length in the same thyroidectomized animals was less by 35 per cent than that in their controls.

Summary. The removal of the thyroid glands in albino rats causes a decrease in the secretory action of the pancreas, the change being evident within 2 weeks after the operation, when no compensatory mechanism for the lost glands has yet been evolved.

CONCLUSION

Summing up all the evidences that I have been able to obtain in the course of my investigations into the effect of thyroid-feeding as well as thyroidectomy upon the secretory action of the pancreas, I may state the following conclusions: The secretory action of the pancreas varies considerably according to the amounts of thyroid-secretion circulating in the body. A moderate augmentation of the thyroid-secretion in the body provokes the hyper-functioning of the pancreas, whereas a lack of the thyroid secretion induces a reduced activity of the gland. As to the part played by the thyroid-secretion in the normal functioning

of the pancreas, we may infer that the thyroid-secretion acts as an excitatory autacoid upon the pancreas in maintaining its normal activity.

Further investigations must decide the question how the excitatory effect of the thyroid-autacoid upon the secretory action of the pancreas differs from that of *secretin* which has generally been regarded as an excitatory hormone acting as a specific stimulus to the pancreas-cells—Bayliss and Starling (1). It has long been a generally accepted theory that the thyroid-secretion acts as a stimulus to oxidation. Furthermore, according to Herring (4), the thyroid-secretion stimulates the suprarenal glands and causes them to produce increased amounts of adrenalin. Koopman (5), moreover, found that the thyroid-autacoid can cause an increased formation of antibody in the serum. A similar excitatory effect we see now in its action upon the secretory function of the pancreas. In view of these evidences, it may be concluded that the thyroid hormone does not act as a specific stimulus to a particular organ or a particular physiological function, as does the *secretin* to the secretory function of the pancreas, but that it probably acts as a non-specific stimulus generally upon various physiologic functions of the animal-organism.

The experimental work was done in the laboratory of Professor Miura's Clinic. I am much indebted to him for his care and supervision. My hearty thanks are due to Prof. M. Kojima (in the Naval Medical Academy) for his help.

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A STUDY OF WEIGHT REGULATION IN THE ADULT HUMAN BODY DURING OVER-NUTRITION

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In contrast to the brilliant successes that science has won in the study of nitrogen equilibrium, there is a rather discouraging inconclusiveness in the work that has been done on the factors that regulate the nutritive balance of the fat tissue of the body, and the balance between the total intake and total output of potential and kinetic energy in the adult warm-blooded organism. We do not yet know what mechanism there is to prevent the unlimited accumulation of potential energy in the form of an overload of adipose tissue. Is nerve regulation through changing appetite the only guide, or does the body vary its destruction of fats and carbohydrates in accordance with their fluctuating intake, somewhat after the manner that it varies its nitrogen exchange?

The best road to a true solution is probably in a study of selected contrasting individuals of the over-fat and under-fat body habits, or better still, of the easily fattening and difficultly fattening types.

The problem was first drawn to my attention by observation of myself, and the experiments here reported were performed upon myself as a selected example of the "spare" or apparently non-fattening type. I had long noted my inclination toward a very copious diet of predominantly starchy nature, in spite of which my weight remained fairly constant, even on a moderate round of activity, at a figure well below the average for my stature. If the hereditary constitution is important in this connection, such family data as I possess seem to indicate that I am derived largely from non-fattening strains.

It has long been recognized that food intake has a powerful effect on the rate of oxidation in the body. In the case of protein food, Rubner (1) distinguishes two such effects. The first of these is the oft discussed specific dynamic effect. The second is a change which appears

more gradually, and is explained by him as a stimulus from plethora of nitrogenous products in the cell fluids. The specific dynamic effect occurs equally in well-fed and badly-fed animals, and so it cannot function as a safety valve for excessive intake. But the secondary or plethora effect may very well so function. According to Rubner, this effect shows itself as a cumulative increase in the specific dynamic effect of heavy protein meals, when they are administered on a series of days. He states that in spite of its cumulative character, this secondary effect is not in evidence during the hours when no food is being absorbed. Consequently a dog that has been over-fed in this manner shows no change in its basal metabolic rate, as ordinarily determined on an empty stomach (p. 260, loc. cit.).

Similar results are reported by Dengler and Meyer (2) in their study of the basal metabolism of a man who was over-fed with protein. They found the basal rate changed to a surprisingly slight degree as a result of nitrogen accumulation, and hence conclude that the excess of stored nitrogen was in a non-stimulating form.

A. Müller (3), in tests on a young man, found that the secondary rise on a high protein diet develops rapidly, before much nitrogen storage can have occurred, and then fails to keep pace with the stored nitrogen, and is at best trivial in proportion to the quantity of nitrogen that is finally accumulated. So he does not look upon this heightened catabolism as a result of the stored nitrogen.

Grafe and Koch (4) experimented upon persons who entered a clinic in an extremely under-nourished condition. The two principal subjects were both put through a heavy feeding period of 7 weeks' duration to bring them back to normal weight. There resulted a very notable increase in the gaseous exchange. Thus the adult subject, at the initial weight of 40.3 kgm., showed a basal metabolism of 1081 cal. (26.8 cal. per kgm.) and a dynamic effect from eating a meal equal to 24 per cent of the calorie value of the meal. Seven weeks later at a weight of 60 kgm., the basal metabolism had risen to 1946 cal., (32.3 cal. per kgm.) and the dynamic effect had risen to 32 per cent of the fuel value of the meal. Most of the gain in basal metabolism occurred near the middle of the experiment. Although it appeared less rapidly than in Müller's experiment, it was of greater magnitude. The tests of the dynamic effect of the mixed diet fluctuated widely, and cannot be easily interpreted.

Atkinson and Lusk (5) experimented along lines similar to those followed by Rubner, but by over-feeding to a greater extreme were

able to report that prolonged and excessive meat diet can cause a rise of the dog's basal metabolism which lasts for as much as two and one-half weeks after the special diet is ended. For their case, then, they conclude that the excess nitrogen was accumulated in a stimulating form.

The converse to the idea of increased or spendthrift oxidation during over-nourishment, is the idea of an especially economical oxidation during under-nutrition.

Anderson and Lusk (6) studied the respiration and the treadmill efficiency of a dog working with empty stomach, with definite meals, and during a prolonged fast. Among other things it was found that the markedly lowered basal metabolism of the fasting period, with its economy of nourishment, carried over into the period immediately following the fast. Eighteen hours after the first liberal meal the metabolism was at essentially the same base level as during the fast, showing that the total condition of the body, and not the question of a large or small influx of food on the previous day, determined the height of the basal metabolism. It is thus to be noted that this dog showed a persistent economy of metabolism after a period of fasting, comparable to the period of wasteful metabolism which Atkinson and and Lusk's dog showed subsequent to a course of over-feeding.

The period of the World War has brought out a series of papers on gaseous metabolism during prolonged under-nutrition. Zuntz and Loewy (7), (8), who have a series of records of their basal metabolism since 1888, studied the effect on themselves of eating the German war ration, practically without any of the additions that free use of money could furnish. On this diet, which was inadequate in fuel value, and especially deficient in protein, they report in the case of Zuntz a 10 per cent fall of basal metabolism below the previous average, and in the case of Loewy a fall of 16 per cent. This decrease is reported in terms of calories per day per square meter by the Meeh formula. The more accurate DuBois formula would show even greater percentage changes in rate. Muscular efficiency on the treadmill had gone down in comparison to previous tests.

F. G. Benedict, Miles, Roth and Smith (9) worked on the metabolism of volunteer squads of young men during experimental under-nutrition which covered three months of low diet. They found a very remarkable fall in the total fuel needs. Starting at an unknown high level which was above 3000 cal., and may have been as high as 3800 cal., they were finally able to hold their weight constant on an average net intake per

person of 1950 cal., the fecal and urinary calories being estimated and deducted. Meanwhile there had been a notable loss of nitrogen, 130 grams previous to the first serious interruption (at Christmas) and further losses to the end of the experiment. The basal metabolism fell from an average of 1686 cal. at the start to 1367 cal. at the lowest point, or from 940 per sq. m. to 788 per sq. m. Thus the absolute figures fell 20 per cent and the rate per square meter came down 16 per cent. A remarkable slowing of the pulse accompanied this change. Efficiency in the tests of mechanical work was not impaired. The authors ascribe the changes to the withdrawal of the influence of dispensable nitrogen from the body, and argue that no great inroads had been made on the essential protoplasm.

Joffe, Poulton and Ryffel (10) report upon a case of extreme undernutrition in a vegetarian, who had probably previously habituated himself to a very meager intake. Throughout the tests the basal rate stayed in the neighborhood of 26.6 cal. per square meter per hour, or 638 per square meter per day, calculated according to DuBois. The increment of oxidation during work was about average, showing that the man had about the average of calorie efficiency in work. His pulse was always below 50 in the reclining position.

Investigators agree, then, that when the diet is varied downward there is a factor or group of factors tending to adjust the calorie output to the intake.

All the researches thus far reviewed follow the method of gas analysis, and judge the balance between intake and output so far as possible by a direct measurement of both. Another plan of procedure is to depend upon the body weight as the criterion to show whether a balance of intake and output has been established. In order to obtain convincing results by this plan it is necessary to let the tests cover long periods of time, and also to have very large differences in the measured diets of the different experimental periods, so as to far outweigh any variations in energy expenditure that may come from uncontrolled factors. In the past this general mode of experiment has been used, either with or without a supplementary study of the gas metabolism, chiefly by Neumann and by Grafe and his pupils.

Neumann (11) carried out upon himself one of the most prolonged quantitative diet experiments ever recorded, and showed a food intake which gave averages on different years of 2427 cal. and 2057 cal. respectively per 70 kgm. body weight. The actual weight in the former test (1895–96) averaged 66.5 kgm., and in the latter test (1900–01)

averaged 72 kgm. In both years the weights were virtually stationary, tending slightly upward. Neumann's results seem to show ability of the organism to stabilize its weight on either low or medium intakes of fuel. But they do not deal in extreme differences of diet.

Grafe and Graham (12) carried out a prolonged experiment upon a dog, keeping full account of the nitrogen metabolism and weight during very marked over-feeding. From time to time the gas exchange was also determined. Although prevented from taking much exercise, this dog showed extraordinary constancy of body weight during both normal diet and excessive feeding. A puzzling feature is the fairly moderate gas exchange which the dog showed in all the tests.

It might be hoped that comparison of the metabolism in abnormally obese individuals and in persons of normal body habit might throw some light on the factors that prevent most persons from fattening indefinitely. This aspect of the problem was studied by Rubner (13), by A. Magnus-Levy (14) and by von Noorden (15). As summarized by von Noorden, the weight of evidence in these earlier papers is for a fairly comparable metabolic rate in these and the normal cases. DuBois and his colleagues improved this observation by applying new methods for determining the surface area of the human body. (See James H. Means (16), and F. C. Gebhart and Eugene F. DuBois (17).) Using DuBois' determinations of surface, it is easily shown that the great majority of over-fat subjects have a basal rate falling well within the normal rate per square meter of surface. This establishes the fact that the laying on of fat is not caused by a depression in the basal rate. The alternatives still left to account for an obese human type are *a*, an unproved possibility, referred to by von Noorden, that without having a lowered basal rate, the obese may still show an exceptionally low cost of digestion, perhaps by not showing the full normal specific dynamic effect of foods; or *b*, that they partiany or entirely lack Rubner's "secondary effect," which causes an upward shoving of the specific dynamic effect, and sometimes even of basal metabolism, whenever the protein over-nutrition becomes cumulative; or *c*, that control over fattening is not referable to any alteration of basal metabolism nor of the energy cost of digestion, but is to be sought in some such factor as a changed appetite.

We may sum up from the literature that under-nutrition (with loss of nitrogen) has a marked lowering effect on the basal metabolic rate and on the total metabolism of the twenty-four hours. Over-nutrition, coupled with heavy enrichment of the body with nitrogen, has at

least in some instances an effect on the basal rate, and has been repeatedly found by Rubner and others to push up the specific dynamic or stimulating effect of protein during absorption, to higher and higher figures. Whenever these factors are at work they all tend to limit the fluctuation of the body mass, and especially to limit the accumulation of body protein. Grafe and his collaborators are the only ones who have argued that a similar type of factors is powerful in preventing the immoderate accumulation of body fat.

Outline of experiments. The general intention of the experiments here reported was to attempt to establish constancy of weight at various levels of total intake. In order to insure the adequacy of the protein and accessories throughout the experiment, milk and eggs figure in all the dietaries used. Small amounts of fats were used but excesses were avoided because it is too easily conceivable that fats might be shunted into the adipose tissue, making a passive increase of body weight without in any way having shared in the metabolism. The main source of nourishment was carbohydrate, from rice, wheat and oats, and the experiments consisted chiefly in varying the quantity of starchy food from these sources.

The first test in March, April and May, 1916, was to find the minimum diet that would maintain an approximately normal body weight. After that the intention was to establish a constant weight on a high level of exchange. This attempt lasted with interruptions from May, 1916, to July, 1917.

At the height of this period of maximum weight and food intake the basal metabolism was determined by the analysis of gas exchange, conducted at the Carnegie Nutrition Laboratory, through the courtesy of Dr. F. G. Benedict and Dr. Thorne M. Carpenter. The body weight was then brought back rather abruptly to the initial level by means of a low calorie diet in July and August, 1917. To conclude the experiment another determination was made of the quantity of food necessary to hold the weight constant.

During several of the above periods records were made of general physical activity. Data were taken for the nitrogen balance during the period of rapid reduction. Analyses of foods were made for this period, but not for any of the other periods, nor were any tests made of combustion value. Instead the diet was limited to a very small selection of foods that would be as free as possible from erratic fluctuations in composition. This method is undoubtedly liable to a certain degree of inaccuracy, but not, we believe, to major errors

TABLE 1
Condensed outline of dietary

TABLE 1

Condensed outline of dietary

	EXPERIMENTAL PERIOD AND DATE								
	I	II		III and IV		V		VI	
	1916 March 4 to 29	1916 March 30 to April 22	1916 April 23 to May 9	1916 May 10 to June 13	1916 June 14 to October 23	1916 October 24 to December 12	1916 December 13 to March 23	1917 March 24 to June 4	1917 June 5 to July 7
Rolled oats, gram (air dry)	0	0	0	0	Ate heartily of varied and liberal diet; outdoor life for 2½ months.	80	On training; under-slept, under-ate, and lost weight at first, then regained the weight gradually on a liberal diet.	100	Diet irregular but essentially like Period VI. (Respiratory analyses).
Rice, gram (air dry)	300	300	300	325		200		200	
Milk, cc	1450	1450	1450	1450		1450		1450	
Butter, gram	30	30	35	50		50		50	
Rolls (40–50 grams each)	Two	Two	Two	Six to ten		Six to eight		Six to eight	
Eggs (45–55 grams each)	Two	Two	Two	Two		Two		Two	
Sugar, gram	0	0	0	30 grams some days		0		0	

	EXPERIMENTAL PERIOD AND DATE									
	VII	VIII		IX					X	XI to XIII
	1917 July 8–14	1917 July 15–25	1917 July 26 to September 1	1917 September 2–5	1917 September 6–8	1917 September 9	1917 September 10 to October 3	1917 October 9–12	1917 October 13–23	1917 October 24 to November 27
Rolled oats, grams (air dry)	100	65.9	30	47.5	100	100	100	100	100	100
Rice, grams (air dry)	200	96.4	60	95	200	200	200	200	200	200
Milk, cc	1402.7	1541.9	1417.5	1402	1393	1393	1393	1431	1431	1431
Butter, grams	40 (pure fat)	0	0	0	0	0	0	0	0	50
Eggs (45–55 grams each)	100.2 gm.	251.1 gm.	251.3 gm.	126.5 gm.	Two	Two	Two	Two	Two	Two
Shredded wheat biscuit (about 30 grams each)	375 gm.	6.8 gm. (av.)	0	0	0	One	Two	Three	Four	Four

or large systematic discrepancies that would alter the tenor of the conclusions.

The figures for the calorie values of the foods are taken consistently from Atwater and Woods' tables published by the U. S. Dept. of Agriculture (18). These figures are based on Rubner's standard values for the physiologically available energy in protein, fat and carbohydrates when presented in the digestive tract in "perfectly digestible" form. Up to July, 1917, the foods were all of this "perfectly digestible" form, and it would be correct theoretically to use these fuel values without deducting for the loss by feces. After that date the use of shredded wheat in the diet caused a considerable increase in the moist weight of feces, but apparently not so great a change in the dry weight. The dry weight of a series of feces samples which were closely comparable to those of March and April, 1916 (ration of 2744 cal.), averaged 27 grams per day. This figure comes from an unreported preliminary test made in April, 1915. In June, 1916, it was about 51 grams per day (on 3800 calories), and in July, 1917, it was 78 grams (on 4113 calories with much shredded wheat). In August, 1917, it was about 22 grams (low diet), and in November about 35 grams (3200 calories with medium supply of shredded wheat).

The principal data obtained are the records of weight variation. Every effort was made to obtain reliable and comparable weighings. The regular hour was between 11:30 a.m. and 12:00 m. The fewest possible changes were made in the dietary of the breakfasts, in order to minimize the fluctuations that come from variation in the contents of the digestive tract. Defecation ordinarily occurred in the forenoon, and no weighings are included from the exceptional days on which it had not occurred before the hour for taking the weight. No laxative was used at any time. Care was taken that the bladder should be empty and that the stomach should not contain drinking water at the weighing hour. Whenever the weather was sultry, some water was drunk at about 9:30 to insure against shortage at 11:30. In spite of these precautions there were some rather disconcerting fluctuations of weight, that must be ascribed to variations of water metabolism. Some of the low weights that show abnormally low water content occurred in connection with temporary constipation, and were thus automatically excluded. In cases of insomnia (of which there were several instances toward the end of the experiments) there always resulted an abrupt transitory fall of weight, which is probably chiefly due to an accelerated renal activity. Some of these weights were

TABLE 2

Relation between food intake and body weight

	PERIOD AND DATE												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
	1916 March 4–29	1916 March 30 to May 9	1916 May 10–25	1916 May 26 to June 12	1916 October 24 to December 12	1917 March 24 to June 4	1917 July 8–14	1917 July 15 to September 1	1917 September 2 to October 12	1917 October 13–23	1917 October 24–27	1917 October 28 to November 10	1917 November 11–25
Duration in days	26	41	16	18	50	73	7	49	41	11	4	14	15
Initial weight	63.89	62.11	61.60	63.85	70.05	71.20	74.08	74.72	65.14	62.31	61.75	62.00	61.43
Average weight	62.90	61.80	62.76	64.89	70.78	71.81	74.28	68.90	63.75	61.94	61.88	61.49	61.32
Average calorie intake	2733	2744	3480	3806	3376	3545	4113	1874	*2441	2781	3183	2970	3204
Deviation of weight from period II	+2%		+2%	+5%	+13%	+16%	+20%	+11%	+3%	+0.2%	+0.1%	−0.6%	−0.8%
Deviation of body surface from period II	+0.7%		+0.6%	+2.1%	+5%	+7%	+8%	+5%	+1.3%	+0.1%	+.04%	−0.3%	−0.3%
Deviation of calorie intake from period II	−0.4%		+27%	+39%	+23%	+29%	+50%	−32%	−11%	+1.3%	+16%	+8%	+17%
Average daily change in weight	−0.68	−0.012	+0.141	+0.129	+0.030	+0.024	+0.091	−0.196	−0.069	−0.050	+0.063	−0.044	−0.003

recorded, but care was taken not to make unsuitable use of those particular figures. An abnormally low weight (500 grams below the previous day) was found on November 27, 1917, at the onset of a heavy nasal catarrh. It was discarded, leaving November 26 the last valid weighing. No weights were discarded for any other causes.

It must not be forgotten that body weight is only an approximate criterion for the equality of intake and output. A person who is oxidizing as much as 100 calories (11 grams) of body fat a day, may replace enough of that fat with water and have enough other fluctuations of his water metabolism to completely mask the fact for many days. This makes it very necessary for experiments upon the body weight to cover long periods of time.

Low level tests of 1916. The initial low level tests are not to be understood as representing subnormal nourishment or any great degree of emaciation. At that time (age thirty-four) my weight had been fairly constant for a number of years, between 64 and 66 or possibly 66.5 kgm. This is below the average for the height of 181.2 cm., but if allowance is made for a rather short and narrowly built trunk, it does not necessarily indicate under-nutrition.

No record of activity was made during this period, but the round of occupations corresponded very closely to the recorded activities of May and June, 1917, the schedule of duties, the plan of life and the daily habits being almost identical. This means about five miles a day by pedometer, about forty-five meters of staircases climbed per day, and a variety of light occupations connected with teaching and laboratory, many of which are already included in the estimated mileage. Night hours and habits of sleep were good, averaging not far from 8.2 or 8.3 hours rest per night. It should perhaps be mentioned that I am not a quiet sleeper. By fastening a pedometer to one ankle, it was found that in an average night 150 to 160 movements would be made, of sufficient vigor to be counted as steps by the pedometer. No sports, muscular games or pleasure walks were indulged in during any of the experimental periods upon measured diets. On this quiet manner of life, starting at a weight (on March 3) of 64.16 kgm., it was attempted to establish a nutritive balance at about 2725 calories. This diet was probably a little less than was eaten before the experiments. As long as it lasted the sensations of hunger before meals were more than customarily acute.

For the entire duration of the test, till May 9, the tendency to lose weight was not entirely overcome. The 41-day period, March

30 to May 9, 1916 (period II), shows an average daily loss of 12 grams on 2744 calories of food, at an average weight of 61.81 kgm. Thus the body requires more than 2744 calories to sustain it at 62 kgm. even under the very moderate conditions of activity. Assuming that the 12 grams of flesh lost had a calorie value not higher than fat, the daily expenditure was more than 2744 and less than 2855 cal. A similar calculation from the last seventeen days of this period gives an expenditure of more than 2753 and less than 2827 cal.

These figures are distinctly larger than the ordinary expectation at the indicated degree of activity. Based on a height of 181.2 cm. and a weight of 61.8 kgm., the expected output of energy can be listed approximately as follows:

	calories
Basal metabolism, 24 hours[1]	1593.0
Added for 5.8 hours sitting, $\frac{5.8}{24} \times 0.1 \times 1595$	38.5
Added for 10 hours standing, $\frac{10}{24} \times 0.2 \times 1595$	132.1
Added for walking 5 miles, $5 \times 0.6 \times 65.4$ (i.e., clothed weight.)	197.2
Added for estimated activities that fail to show on the pedometer (50 per cent of the walking)	98.6
Added for climbing 45 m. of stairs,[2] $45 \times 4 \times 65.4 \times 0.002343$	27.6
Added for descending 45 m. of stairs,[3] $45 \times 2 \times 65.4 \times 0.002343$	13.8
	2100.8
"Digestion cost" of 2744 cal. food (6 per cent)[4]	164.6
Predictable calorie output	2267.4
Food eaten	2744.0
Discrepancy of intake over predictable need	476.6
	or 21 per cent

Where some of these items are problematical, they have been estimated at a rather liberal rate, e.g., the ten hours standing and the unknown activities equivalent to 2.5 miles of walking. The "digestion cost," and the increments of metabolism due to the sitting and standing positions have been placed at figures that may appear rather low, because the data upon these factors published in recent years by F. G. Benedict and his collaborators seem to call for a moderate estimate

[1] Harris and Benedict's prediction for height, 181.2 cm.; weight, 71.81 kgm

[2] Assuming a mechanical efficiency of 25 per cent.

[3] Assuming the descent to cost half as much as the ascent.

[4] Benedict and Carpenter (22).

of their effect. (See Benedict and Murschhauser (20); Benedict and Carpenter (22).)

The failure to overcome the negative balance on an intake of 2744 calories indicates a physiological tendency, even at low weight levels, to expend more energy than is to be expected from the list of external activities. This physiological wastefulness left the system slightly under-nourished on a diet which ought otherwise to have been more than adequate.

High level tests. May, 1916, to June, 1917. Periods III and IV of the experiments, covering May 10 to June 13, inclusive, differ from the preceding in an increased amount of carbohydrate with a slight increase of butter (50 instead of 35 grams). In order to avoid overwhelming the stomach with an excessive volume of food, the additions were chiefly to the bread ration rather than the moist cereals. For two weeks (period III) a diet of 3480 cal. was maintained, on which a gain was made of 2.25 kgm. As there was no promise of reaching a balance quickly, a further increase of bread was made and the high feeding was kept up till June 13 (period IV, May 26 to June 13, inclusive). With an average diet of 3806 calories, the daily gains continued about the same, to the final weight of 66.17 kgm.

Periods III and IV can count only as preliminary, being too short to overcome the doubts that are necessarily caused by the fluctuation of water metabolism. It is well known (Bischoff and Voit (23); Voit (24) discussion on page 347) that heavy feeding with a very starchy diet will lead to a notable accumulation of water in the tissues. But we have no basis on which to predict the extent or duration of this process, and so have hardly any clue to the calorie value of the accumulated weight in our experiment. If the accumulation during period IV had been exclusively pure fat,—an assumption which is certainly contrary to fact,— it would have represented a fuel accumulation of 1200 calories per day, or more than the total daily excess of food in calories. The high diet needs to be continued long enough to more nearly stabilize this factor of water intake.

The summer, from June 14 to October 23, was on a liberal and hearty diet. Butter, meat, milk and especially all forms of carbohydrates were supplied unstintingly, and were intentionally taken to the full limit that could be relished. For 2½ months the appetite was under the stimulus of an active outdoor life. The resulting weight of 70.05 kgm. on October 24 was considerably the largest that I had ever reached.

There then followed a measured period of fifty days (period V) averaging 3376 calories per day, and with daily activity at most only slightly greater than in the other experimental periods. The average gain during the fifty days was 30 grams per day. This must be interpreted as showing a genuine plus balance, because the gain is distributed throughout the period, including its latter portion when water equilibrium must have been reasonably well established. The small size of the daily gains makes it seem probable that the fuel cost of maintenance and activities had risen along with the body weight.

The period of over-nutrition was unavoidably interrupted in the winter of 1916–1917 by a season of heavy duties, impaired sleep and consequent intolerance for an excessive diet. But there was no interruption of the record of essentially good health, and in February and March good nutritive conditions made it possible to recover the greater part of what had been lost.

On March 24, 1917, period VI was started with the initial weight of 71.20 kgm. It was continued 73 days with an average intake of 3545 calories of food and showed an average gain of 24 grams per day. As in the case of the preceding period, the weight curve does not suggest any modification of the water metabolism by the carbohydrate food. Its greater length renders it a comparatively safe period on which to base calculations. With an average weight only 1.03 kgm. above that of period V the additional 170 calories are carried with a smaller daily gain. There seems, then, to have been a definite although not very great growth of that extravagance in use of fuel food which was noted during period II.

This high expenditure of fuel food is much more pronounced in this period than in any previous test. This is not explained by activity, as the daily habits of period II and period VI are the nearest imaginable approach to duplicates of each other. It is likely that period V had slightly, but only slightly greater physical activity, on account of a slightly heavier schedule of university duties. In that case the relative metabolism of period VI above period V becomes the more noteworthy. Period V is the only one of the first six periods that differed perceptibly in its round of activities.

The record of activities with the aid of pedometers began to be taken that spring, and included about four weeks of period VI. Three pedometers were worn at the start, one at the belt, a second at the right ankle, intended to show minor movements of locomotion, and the third at the right forearm. The forearm position proved to be use-

less, because of the changes of posture, and because sudden motions were liable to jam the bob of the pedometer and make it stop recording. The ankle pedometer was also given up eventually. It seemed to be able to record more motions than the belt pedometer during the sitting and standing occupations,but it suffered from very nearly the same mechanical difficulties as the one on the arm. It was read night and morning for 14 days, and supplied the data on restless sleeping to which I have already referred. The belt pedometer was worn only during the waking hours, and was read once daily regularly through the remainder of the series of experiments.

The estimate of stairs climbed is a fairly accurate average, based on the extremely uniform round of places visited each day.

The record of hours devoted to sleep did not commence till July of that year. They vary from 8.20 to 8.35 hours in different months. I believe it probable that in periods I to VI the hours were not less than the latter figures, but in order to insure against an under-estimate of activities, I have calculated on the basis of 8.2 hours.

A rather problematical point as regards activity is the number of hours in the standing position, and the amount of activity of gentler type than would record on the pedometer. Much time was spent standing, as I made but little use of chairs in the laboratory. In order to insure against an under-estimate, the figure has been set at ten hours for periods I to VI inclusive. In the same spirit I have assumed an arbitrary figure of half the energy of an average day's walking to cover the undeterminable lighter activities. These data give us the following estimate of the daily energy output that would fulfil the ordinary expectation for this period:

	calories
Basal metabolism, 24 hours[5]	1724.0
Added for 5.8 hours sitting, $\frac{5.8}{24} \times 0.1 \times 1724$	41.7
Added for 10 hours standing, $\frac{10}{24} \times 0.2 \times 1724$	143.7
Added for walking 4.82 miles, $4.82 \times 0.6 \times 75.4$ (i.e., clothed weight)	218.1
Added for activities not shown on pedometer (estimated as 50 per cent of the walking)	109.0
Added for climbing 45 m. of stairs,[6] $45 \times 4 \times 75.4 \times 0.002343$	31.8

[5] Harris and Benedict's prediction for height, 181.2 cm.; weight, 71.81 kgm.

[6] Assuming a mechanical efficiency of 25 per cent.

Added for descending 45 m. of stairs[7]	15.9
	2284.2
"Digestion cost" of 3545 cal. food (6 per cent)[8]	212.7
Predictable calorie output	2496.9
Food eaten	3545.0
Deduction for excess feces (21 grams excess above the normal 30 grams) 21 × 6.2 cal.	130.2
Net cal. from diet	3414.8
Discrepancy of intake over predictable need	+37 per cent

This diet seems, from the calculation, to be no less than 37 per cent in excess of the predictable need, while the diet in period II showed an excess of 21 per cent. But it is necessary to allow for the difference between a falling weight in period II and a rising weight in the later period. If we assume that the calorie value of the flesh gained and lost does not exceed that of pure fat, then the total oxidation of material (food and body fat) in period II lies 21 per cent to 23 per cent above the predictable figure, and the oxidation in period VI is between 27 per cent and 37 per cent above the prediction. *The discrepancy between the predictable and the observable expenditure has undergone an absolute increase and probably even a relative increase.*

Basal metabolism during over-nutrition. In June, 1917, the courtesy of Dr. F. G. Benedict and of Dr. T. M. Carpenter and their collaborators in the Carnegie Nutrition Laboratory supplied me with three determinations of my basal metabolism by their usual routine methods. On June 13 and 16 the tests were in the bed respiration chamber, Miss Corson in charge, at the Deaconess' Hospital, Boston, and on June 20 by means of the large Tissot spirometer and Haldane gas analysis methods, carried out by Doctor Carpenter. These tests were all of them on the high diet, the average intake of the three 2-day periods preceding these three tests being 3965 calories. As the experiments necessarily interrupted the diet, being taken at breakfast time on an empty stomach, and not being concluded for some hours, the average intake for the whole period of June 9 to 19 inclusive is only 3790 cal. On this diet of essentially 3965 calories representing the maximum in take up to that date, and at a body weight of 73.62 kgm., the recorded metabolism is very uniform with the single exception of the first respira-

[7] Assuming the descent to cost half as much as the ascent.

[8] Benedict and Carpenter (22).

tion period of the first day, the period of introduction to the apparatus, when the psychic effects undoubtedly militated against complete relaxation. Rejecting this half-hour period, the average of the other results, by indirect calorimetry from the gas analysis, are 73.32 cal. per hour, or 1762 cal. per day for the waking basal metabolism. The prediction for weight 73.6 kgm., height 181.2 cm. and age thirty-five, made by Harris and Benedict (19) is 1749 cal., constituting an almost perfect agreement with the finding.

A very high respiratory quotient should be noted; the figures being 0.94, 0.93 and 0.89 on the three different days. In the first two of these cases the non-protein respiratory quotient was 0.98. Thus even thirteen to fifteen hours after the last meal, and although fats were by no means excluded from the high carbohydrate diet, the oxidative processes were limited practically to carbohydrate and protein.

In spite of this fact that the high carbohydrate of the previous evening is still exerting a great influence upon the respiratory quotient, the metabolic rate conforms exactly to the prediction for the basal or post-absorbtive rate. The prolonged diet has *not raised the basal metabolic rate above the normal average.*

As I believe the figures have demonstrated that the metabolism as a whole is extravagant above the average, we shall have to look for the element of extravagance in some other factor than the basal rate.

Return to normal level. The return to a normal weight was accomplished in the summer months of 1917, while at the laboratories of the University of Illinois Medical School. During this period the food, urine and feces were analyzed for the determination of nitrogen balance. The urine analyses were continued most of the time to the end of the experiments on November 26, and the weighed diet up to that date was limited to the same set of foods as were used in the summer period. Thus the nitrogen exchange was followed in full or in part during the whole of these $4\frac{1}{2}$ months. Table 3 summarizes these analytical data.

The diet differed from that of previous periods in the substitution of shredded wheat in place of the more difficulty analyzable white rolls, and also in the change from soft boiled eggs to a form of custard, which could easily be sampled for analysis when mixed and strained and ready to cook. Clear centrifuged butter fat was used in the summer months in place of commercial butter. Head rice, Quaker brand rolled oats and whole milk completed the diet. The analysis of the milk was by taking equal daily samples. The shredded wheat was

TABLE 3

Summary of nitrogen exchange

	PERIOD AND DATE												
	VII		VIII				IX			X	XI	XII	XIII
	1917 July 8-9	1917 July 10-14	1917 July 15-24	1917 July 25 to August 7	1917 August 8-29	1917 August 30 to September 1	1917 September 2-12	1917 September 13 to October 8	1917 October 9-12	1917 October 13-23	1917 October 24-27	1917 October 28 to November 10	1917 November 11-25
Duration in days	2	5	10	14	22	3	11	25	4	11	4	14	15
Dietary N	20.26	19.74	15.68	13.60	13.42	13.50	13.37	14.60	15.13	15.71	15.88	15.05	15.97
Urinary N		14.30	16.02	14.16	13.23			12.17		12.80	12.09	11.13	12.16
Fecal N		3.54	1.59	1.27	1.02								*2.00
Daily N. balance		+1.89	−1.93	−1.82	−0.83								+ 1.81
N. balance for period		+9.47	−19.34	−25.53	−18.35								+27.07
			Loss in 46 days 63.2 gm.										
Calorie intake	4127	4110	2212	1811	1772	1788	2198	2537	2659				
Per day	av. 4115		av. 1874				av. 2441			2781	3183	2970	3204
Change of weight during period	+ 0.64		−9.58				−2.83			−0.55	+ 0.25	−0.57	−0.05
Change of weight per day	+0.091		−0.196				−0.069			−0.05	+0.063	−0.044	−0.003

* 12.01 grams in 6 days, November 14–19, inclusive.

broken small and mixed thoroughly in a large, moisture-proof container, from which the daily portions and the samples for analysis were taken. The rolled oats and the rice were similarly mixed and sampled. All these cereal samples were ground before dividing them into smaller portions for the analyses. The Kjeldahl-Gunning method was used in all the nitrogen determinations.

The first week of this summer series, on a diet of 4115 cal., represents very nearly the maximum capacity of the subject for continuous consumption, unless fats were to be used more freely. A substantial plus balance is shown both in nitrogen and in body weight. The period is too short to indicate the extent to which excess oxidation may have developed, but it is clear that the process had not gone far enough to prevent further fattening.

Following this was a period of low nutrition, lasting 7 weeks, with a daily average of 1874 cal. The intention was to dispose of the accumulated body fat without running into the condition of depressed basal metabolism that will occur when there has been a heavy loss of nitrogen. Accordingly the quota of eggs was somewhat increased, and the radical cut was confined to the cereal foods and the butter fat, the latter being entirely discontinued during most of the interval. It was impossible, however, to prevent a strongly negative balance of protein materials, so that the average daily weight loss of approximately 200 grams for the rest of the summer is accompanied by an average nitrogen loss of nearly 1.4 grams per day.

Final equilibrium. The attempts to reach a stable weight during September, October and November make it clear, first of all, that in spite of the considerable loss of nitrogen the body was by no means in a depressed metabolic state. It is impossible to tell whether the body was richer or poorer in protein constituents than it had been during the first equilibrium period (period II) of 1916. After more than a year of high calorie diet, with a fully adequate protein intake at all times, there can be no question that when the diet was changed in July the tissues were copiously stocked with protein materials. The figures obtained after July 15 can be extrapolated so as to show that between that date and the middle of September the body must have lost some 70 or 75 grams of nitrogen,—an amount that probably did not yet leave the body in any greatly depleted condition. When the diet was now increased to near 2500 calorie, this negative balance was checked, or possibly even changed to a small positive figure, but the loss of weight was not entirely overcome. The same was true in the

period on 2780 calories in October, the nitrogen balance being somewhat further improved, but the weight still continuing to decline. A virtually steady weight was at last established on an appreciably higher diet, from October 24 to November 25 inclusive, but not till the nitrogen balance had become definitely positive. Judging from the final fortnight, (period XIII), 3200 calories were now necessary to maintain a body weight of about 61.3 kgm.

During September, 1917, it was impossible to make a satisfactory record of activity, because at that time the exigencies of changing to a new residence caused a temporary increase of manual labor. The October and November records are free from such complications, excepting that the use of a bicycle was commenced at this point, to get to and from work. Habits of sitting and standing were about as in the early months of experimentation. The recorded activities are as follows:

	PERIOD AND DATE			
	X October 13-23	XI October 24-27	XII October 28 to November 10	XIII November 11-25
Duration in days	11	4	14	15
Sleep per day, hours	8.55	8.44	7.78	8.28
Walking per day, miles	4.76	7.62	3.58	4.35
Bicycle per day, miles	2.61	4.48	4.54	4.77
Stairs climbed per day (est.) meters	20	20	20	20

The bicycle route involved no steep grades, and always returned to the level of the original starting point. The bicycle was an exceedingly easy running one, capable of coasting freely on windless days on a gradient of 115 feet (35 m.) per mile, rider plus bicycle having a total weight of 83 kgm.

Without attempting to evaluate the energy used in bicycle riding, I believe it will be conceded to be insufficient to make the activities of the above periods appreciably greater than in periods II and VI. But the irregularities in sleep are probably a rather serious factor, and period XII is probably vitiated for purposes of comparison by the relatively poor "sleep" record. For this reason period XIII seems to be the principal one for a satisfactory comparison with period II, on the assumed basis that they represent essentially the same degree of muscle activity.

The summarized results of the last four periods are as follows:

PERIOD	DATES	INTAKE IN CALORIES	CHANGE OF WEIGHT PER DAY	REMARKS
			grams	
X	October 13–23	2781	−50	Maximum sleep. Activities below average
XI	October 24–27	3183	+63	Excellent sleep. Greater activities
XII	October 28–November 10	2970	−44	Imperfect sleep. Moderate activities
XIII	November 11–25	3204	− 3	Excellent sleep. Moderate activities
I place beside these for comparison the earlier period:				
II	March 30–May 9, 1916	2744	−12	Excellent sleep. Moderate activities

In spite of the inescapable irregularities inherent in human experimentation of this sort, a comparison of the forty-one days in 1916 with the final thirty-three days, both of which were at practically

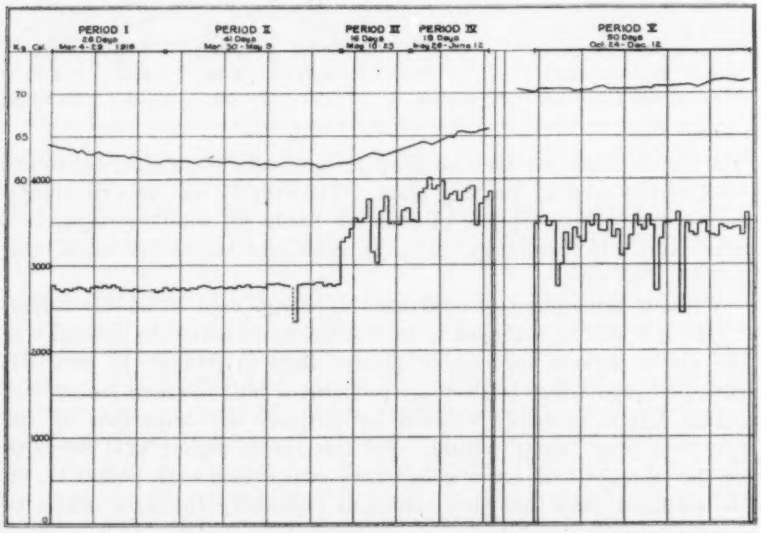

Chart of food intake and body weight, 1916

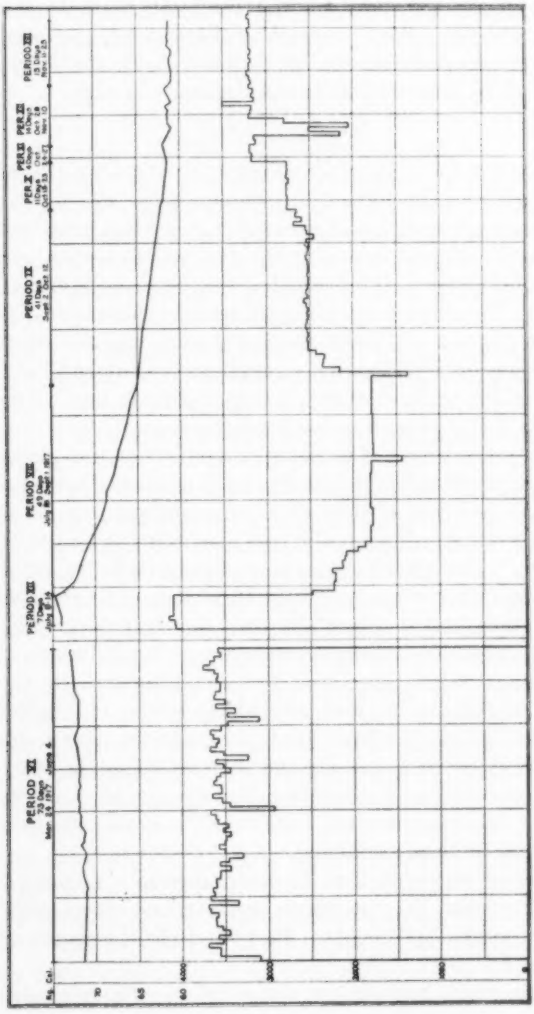

Chart of food intake and body weight, 1917

identical average body weights, brings out very definite evidence of a marked increase in the daily caloric requirement. It is not safe to attempt to calculate exactly how great this change has been, but on the face of the results from the final fifteen days, the daily demand seems to have risen by some 300 to 400 calories.

DISCUSSION

The general results of these experiments are that this example of a person belonging to the difficultly fattening type was found to show a wasteful rate of oxidation during all the feeding experiments, including both the periods in which the diet was moderate or low and those in which a large excess of starch was superposed upon the normal diet. During the prolonged periods of high diet this wasteful oxidation became more pronounced, and it continued so throughout the following periods of under-nutrition, so that even after the body had been brought down again to its original weight, it required more food to keep it at steady weight than had been necessary at the start.

In a preliminary report of these experiments that was made in 1917 (25) considerable uncertainty was expressed as to whether or not these figures could be used as evidence for a compensatory excess oxidation during high feeding. This doubt was based on the fact that a comparison between the nutritive exchange during high feeding and that during the final low-weight periods could only lead to inconclusive results. In the present paper the final low-weight periods are not used for this comparison, and the extravagance of the calorie exchange during over-nutrition is appraised by comparing it with the initial determination of minimum need, as made in 1916. It is believed that this plan of analysis is justified; firstly, because the calorie demand in the final periods is so far above the need as found in 1916 that it seems to show a hangover effect from the months of excess nutrition; and, secondly, because even the initial need is noticeably in excess of the expectation by dead reckoning.

The basal rate of metabolism as determined in a reclining position before breakfast did not rise above the average expectation for the subject's age, weight and height. Pulse and blood pressure were also entirely normal.

It seems clear that throughout the entire experimental series there was some factor at work which caused fuel food to be burned more freely than in the average individual. This factor was not an overactive thyroid as attested by the entirely normal basal metabolism.

It is possible that a part of the waste is attributable to neuromuscular factors. During all the experimental periods the greater part of the daily activities were of the less intense variety, the calorific cost of which is always problematical, because it can never be predicted how much will be wasted in the increased tone of the unemployed muscles. This undeterminable expenditure may easily have varied from the average expectation, and may be responsible for some of the unexplained energy expenditure. But if this were the whole explanation, we ought to find a lessened wastefulness and not an increase during the months of over-feeding when there was a continued stuffed feeling and a disinclination to exertion. For this reason it seems more probable that the main factor is not to be sought in neuromuscular habits, but in some factor in the chemistry of nutrition.

The nitrogen balance may very easily be connected in some way with this problem, for although the actual intake of proteins was never abnormally high, the liberal calorie allowance of the experimental diets and of the subject's previous dietary habits was very favorable to an accumulation of nitrogen. Even the last tests, after there had been a loss of 70 grams or more of nitrogen, may have been under the influence of superabundant stores of protein materials, as that 70 grams were only removed after a maximum storage must have been attained, and by the time that the last experimental weeks had been reached, some of the lost nitrogen had been restored. If the factor causing extravagance is related to this supposed nitrogen enrichment, it is not to be compared with the plethora effect observed by Atkinson and Lusk (5), but it may very possibly be comparable to the "secondary effect" of protein enrichment, which according to Rubner (1) can raise the specific dynamic effect of the food without raising the basal rate. The present experiment differs from Rubner's in that the food for which the specific dynamic effect must be augmented is largely starch instead of protein.

It is also possible that nitrogen enrichment may not be the major explanation of the nutritive condition. For there is still the alternative that von Noorden's (15) suggestion respecting the obese type may have its converse, and the spare type be accounted for by any factor that produces a high "cost of digestion," just as the obese may be supposed to suffer from an abnormally low "cost of digestion." The decision between these alternatives would only be possible after an extension of the tests beyond the limits that have been practicable in the present investigation.

SUMMARY

1. During periods aggregating about three hundred and seventy days on experimental diet, a person of the difficultly fattening type was investigated, first, to determine the minimum food required for maintenance of weight at the customary level; second, to ascertain whether and to what extent an excess of starchy food would be stored by this type of person as adipose in a long period of superabundant measured diet; and third, to ascertain whether after the body was returned to the initial weight with least possible loss of nitrogen, any change had occurred in the minimum requirement of food.

2. The person was found to owe his resistance against fattening to an extravagant calorie requirement which persisted at all times, despite a moderate daily round of activities.

3. This extravagance increased during the course of the excessive carbohydrate diet, and stayed above the initial level even after the return to normal weight.

4. The basal metabolic rate was not involved, but remained strictly normal.

5. The high calorie output and consequent resistance against fattening may find its explanation either in a condition of nitrogen enrichment, or in an upward variation of the "cost of digestion" (and assimilation) of starchy food.

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